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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil - a times series study

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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil - a times series study.

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ABSTRACT

Background SSR (Secondary Sex Ratio) has become an indicator of population balance. Scarce studies have found a direct association of environmental pollution and changes in SSR.

Objectives In order to assess if ambient air pollution in urban areas could be related to alterations in male/female proportion this study objectives to evaluate changes in ambient particulate matter (PM_{10}) concentrations after implementation of pollution control programs in São Paulo city and the secondary sex ratio (SRR).

Design and Methods A times series study was conducted. São Paulo's districts were stratified according to the PM_{10} concentrations levels and were used as a marker of overall air pollution. The male proportion was chosen to represent the secondary sex ratio (SSR=total male birth/total births). The SSR data from each area was analyzed according to the time variation and PM_{10} concentration' areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

Results The exponential regression showed a negative and significant association between PM_{10} and SSR. SSR varied from 51.4% to 50.7 % in São Paulo in the analyzed period (2000-2007). Considering the PM_{10} average concentration in São Paulo city of 44.72 $\mu g/m^3$ in the study period, the SSR decline reached almost 4.37%, equivalent to 30,934 less male births

Conclusion Ambient levels of PM_{10} are negatively associated with changes in the SSR. Therefore, we can speculate that higher levels of particulate pollution could be related to increased rates of female births.

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Article summary

1) Article Focus

- Study the potential influence of air pollution in gender in Sao Paulo in an extended time series period
- Discuss the future impacts of imbalance gender proportionality in urban centers

2) Key Messages

- Air pollution may influence gender determination
- Scarce studies showing this effect in urban centers
- Higher levels of air pollution may be associated to the increase rates of female births

3) Strengths and Limitations.

- We analyzed male/female births in different areas of São Paulo, Brazil.
- We compared areas with different levels of PM_{10} concentration within the city
- SSR varied from 51.4% to 50.7 %, suggesting that air pollution may be associated to changes

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Summary Box

What is already known on this subject?

Air pollution is an environmental risk factor of concern in urban centers all over the world. Reductions in secondary sex ratio has been suggested to be indicative of potential influences of polluted environments on reproductive function. Previous study analyzed the relationship between air pollution and secondary sex ratio in an urban center of a developing country in a restricted time series period.

What does this study add?

The pollution levels in the city has declined significantly due to the national pollution control program since previous evaluation; thus this study extended the period analysis in order to assess if changes in the particulate matter concentration are followed by changes in secondary sex ratio. Data have shown a strong association and could indicate SSR as a potential indicator of population health status orientating future public policies for environmental control.

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Contributorship Statement

The corresponding author has conceived and planned the study design and analysis; all the authors have contributed with the analysis, elaboration and final approval of the manuscript.

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INTRODUCTION

Air pollution is an environmental risk factor of concern in urban centers all over the world. Respiratory and cardiovascular diseases are the most commonly observed and associated effects followed by neoplasia,[1, 2, 3]. However, in the last two decades lesser known effects associated to chronic air pollution exposures have started to emerge [4]. New epidemiological and experimental studies link exposure to reproductive adverse outcomes and investigations have risen different effects to be attributed to air pollution such as low birth weight,[5] miscarriages, [6], preterm birth [7] and decrease sperm quality [8].

Secondary sex ratio (number of male births for every 100 female births) seems to be affected in population living in polluted environments and occupationally exposed to certain chemicals,[9,10,11,12,13]. Although the causality between environmental exposures and declines in secondary sex ration are still controversial, some authors suggest that the SSR as a sentinel indicator of reproductive injury and avoidable health exposures,[14] due to environmental pollution.

Experimental evidence indicate that prenatal exposure to air pollution derived from diesel exhausts is associated with altered sexual differentiation and function,[15]. Studies in humans and animals have found a reduction in the number of male births associated with lower male fertility, but the mechanism by which environmental hazards might change the sex ratio has not yet been established,[9,16].

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In a previous study, we have demonstrated a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}), [16]. This study was conducted in São Paulo Metropolitan Region (SPMR) in Brazil, and the area was divided in terms of level of PM_{10} concentrations. Findings indicated a SSR of 51.7% for the less polluted area whereas for highly polluted area the proportion decreased to 50.7%. This result corresponds to a difference of 1% in total male births, or 1,180 fewer male births in the most polluted regions),[16].

Previous data analyzed a restricted time series period (2001-2003) and during the last years air pollution levels in the city has changed significantly due to the national pollution control program (PROCONVE and PROMOT). In this sense, it is desirable to verify if changes in levels of air pollution are accompanied by concurrent changes in the SSR (male/female proportion) in SPMR. Thus, the purpose of this study was to extend the time period evaluating from 2000 to 2007 to assess changes in ambient particulate matter (PM_{10}) concentrations and secondary sex ratio in the RMSP during this period (SRR).

METHODS

Number of births according to gender

The total number of live births in São Paulo was collected from 2000 to 2007 in a monthly basis representing a sample of 53,612 births. These records were obtained from SEADE, a public foundation which registers population data in the State of São Paulo. The male proportion was chosen to represent the secondary sex ratio (SSR=total male birth/total births).

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Studied Area

São Paulo is the largest Brazilian city, where most important economic activity is concentrated and is responsible for 17% of the country's gross national product. São Paulo is considered the 6th largest city in the world with a population of approximately 11 million in an area of 7,943.82 km² [17].

According to the São Paulo's Environmental State Agency air pollution is derived mostly by vehicles (combustion and re-suspension) and a small industrial contribution. Winter period in São Paulo favors thermal inversions and this may also contribute to non-favorable pollutant dispersion scenario and increased levels of PM_{10} [18]. Air pollution control programs in São Paulo Metropolitan Area are well succeed for the fixed sources however the mobile sources are of government concern.

Air pollution Data

The studied area encompasses the sub-districts where the state environmental agency (CETESB) has air monitoring stations, and where selected according to different air pollution gradients (high and low concentrations' areas). We included districts for which we had a good quality representative data (valid time series) and stratified according to the PM_{10} levels. The districts were aggregated according to the level of PM_{10} concentration as follow: high level ($\geq$40 $\mu g/m^3$) and low level (< 40 $\mu g/m^3$). PM_{10} concentrations were used as a marker of overall air pollution.

In total data were obtained from 5 automatic monitoring stations maintained by CETESB. In all stations, PM_{10} was measured through inter compared beta radiation monitors. The daily values obtained from each station were averaged in a monthly basis and considered as indicative of city-wide pollution levels. There is a correlation

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between PM_{10} concentrations registered at the different sites that means that PM_{10} is regularly distributed along the citywide.

Statistical analysis

The SSR data from each area were analyzed according to the time variation and PM_{10} concentration in the areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

RESULTS

The sub district's average concentrations of PM_{10} in the period ranged from 34.1 $\mu g/m^3$ to 64$\mu g/m^3$ and the SSR from 0.49 to 0.52 as depicted in Fig. 1.

In the less polluted area, the SSR average was 51.4% for 28,022 births recorded whereas in the most polluted area the proportion decreased to 50.7 % for 22,590 births recorded. We observed a general decrease trend in PM_{10} concentrations through the analyzed time period while the SSR simultaneously presented an increase.

An analysis of percentage variations considering the extreme years of the time-series analysis (that is, 2007 compared to 2001) was conducted showing a continuous decrease of PM_{10} concentration associated to an increase in SSR in each monitoring sub-district in the period, except for one monitoring station, which presented the same average level (Fig. 2). Surprisingly, Cambuci (CBC) monitoring station presented no variation in both variables (PM_{10} and SSR); however, this finding confirms the association observed in the other stations where lower PM_{10} concentrations are related to higher SSR.

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The exponential regression showed a negative and significant association between PM_{10} and SSR (Table 1).

Table 1. Bivariate exponential regression analysis and relative risk.

Variable	R²	β	p-value	RR
SSR	0,322	-0.001	0.022	0,999

SSR: Secundary Sex Ratio; RR: Relative Risk

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Fig. 3 emphasizes the inversely relationship of PM_{10} concentrations and SSR, specially from 2002 on, when we can observe the annual variations in both variables occurring in opposite directions, reinforcing the above demonstrated findings.

DISCUSSION

In this study we have evaluated the variation in PM_{10} environmental concentration and SSR in the Metropolitan Region of Sao Paulo, Brazil during the years of 2001-2007. In a previous study conducted in the same area we have noted that there was a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10})[16]. In this study we extended analyzed time period, which allowed us to observe improvements in air quality due to the environmental control politics introduced (motorized vehicles' emissions control) and in the population's gender pattern. Although the air quality increased we still find a significant negative association between the SSR and PM_{10} concentration.

In one region of the city, where CBC station is located (central area of São Paulo city) no variation in PM_{10} was noted and we can speculate that this no variation in PM_{10} is due by the fact that this area has buses emissions as main air pollution source, with lower contributions from cars and motorcycles. No variations in PM_{10} in this region

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shows that air pollution control program have not positively impacted the area leading to the maintenance of the air pollution level. Maintenance of PM_{10} levels was accompanied by maintenance of the SSR for this region. CBC station records and associated SSR can be interpreted as a control unit for other stations where there were variations in PM_{10} concentration meaning that for the same level of air pollution the same SSR was registered.

These results could suggests that there is a possible contribution of PM_{10} levels in SSR variation, explaining more than 30% of the events. If we consider that there is causal relationship the increase of 10μg/m³ in PM_{10} concentration would lead to a decline of 0.995% in SSR. Further, taking into account a PM_{10} average concentration in São Paulo city of 44.72 $\mu g/m^3$ in this study period the SSR decline would reach almost 4.37% which is equivalent to 30,934 less male births.

This behavior (decrease in PM_{10} and increase in SSR) is consistent with previous findings ,[15] that have shown a possible association between exposure to urban air pollution and imbalance of the sex ratio at birth. Other studies have also reported lower sex ratio in residential areas at risk from air pollution from incinerators [19] as well higher sex ratio in areas exposed to polluted air from steel foundry [20].

Potential toxicological mechanisms that might explain and give strength to the environmental contamination causes in the determination of the sex ratio are still inconclusive. There are some suggestions in the literature that include the hormonal status of the parents at the time of conception, differential characteristics and sensibility of sperm of one sex, combination and presence of specific toxic substances (PAH, dioxin) [21,22]. Although we have not evaluated the elemental composition of PM10, previous studies have characterized the composition of these particles from São Paulo city. Chemical elements included Fe, Br, Al, Si, S, Cu, Zn, Pb [23] and PAH such as

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benzene, toluene, etil-benzene e xylene [24]. Toxicological studies have shown that certain toxicants present in ambient air pollution, such as PAH and heavy metals potential endocrine disruptors [25,26].

Increasing differences in the proportion of male/female births can lead, in a mid-long term future, to a deficit in male's population and cause social problems. This scenario gets worst if we consider that male are more prone to premature death because of their trend to engage in risk behavior and violence,[27].

The air pollution control programs (PROCONVE and PROMOT, which refers to emissions limit to new motor vehicles – cars and motorbikes) may have contributed to the improvement in the air quality parameters registered through the decade. Recently, an inspection and maintenance program concerning emissions limits for the old and second handed vehicles was implemented in São Paulo and that may also have favored this scenario. Our findings are important indicators for an advance of the public health endpoints due to the improvement of the air quality in urban centers. Considering the disproportion in the male/female births, this balance is desirable to achieve and maintain in all populations of urban centers. Furthermore, the abatement of air pollution is a target that governments must pursue.

CONCLUSIONS

Although the biological mechanisms responsible for the SSR changes are not clearly established, this study indicate that concentration of particulate air pollution in urban cities are associated with decreased SSR. Also, this data give support for the use of SSR as a potential indicator of the negative health impacts of fuels combustion derived emissions in urban cities.

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REFERENCES

1. Schwartz J.. Long-term effects of exposure to particulate air pollution. *Clin Occup Environ Med* 2006,5(4):837-848.

2. Pope CA, 3rd, Ezzati M, Dockery DW.. Fine-particulate air pollution and life expectancy in the United States. *The New England journal of medicine* 2009,360(4): 376-386.

3. Dockery DW. Health effects of particulate air pollution. *Ann Epidemiol* 2009,19(4): 257-263.

4. Calderon-Garciduenas L, Engle R, Mora-Tiscareno A et al. Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and cognition* 2011,77(3): 345-355.

5. Gouveia N, Bremner SA, Novaes HMD. Association between ambient air pollution and birth weight in São Paulo, Brazil. *Journal of Epidemiology and Community Health* 2004,58(1): 11-17.

6. Mohorovic L, Petrovic O, Haller H et al. Pregnancy loss and maternal methemoglobin levels: an indirect explanation of the association of environmental toxics and their adverse effects on the mother and the fetus. Int J Environ Res Public Health.2010 Dec;7(12):4203-12. Epub 2010.

7. van den Hooven EH, Pierik FH, de Kluizenaar Y et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environmental health perspectives* 2012,120(1): 150-156.

8. Pires A, de Melo EN, Mauad T et al. Pre- and postnatal exposure to ambient levels of urban particulate matter (PM(2.5)) affects mice spermatogenesis. *Inhal Toxicol* 2011,23(4): 237-245.

9. Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. *Emerging Health Threats Journal* 2011;Vol 4 (2011) incl Supplements.

10. Tragaki A, Lasaridi K. Temporal and spatial trends in the sex ratio at birth in Greece, 1960–2006: exploring potential environmental factors. *Population & Environment* 2009,30(3): 114-128.

11. Schnorr TM, Lawson CC, Whelan EA et al. Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environmental health perspectives* 2001,109(11): 1127-1132.

12. Ryan JJ, Amirova Z, Carrier G.Sex ratios of children of Russian pesticideproducers exposed to dioxin. EnvironHealth Perspect. 2002;110(11):A699-701.

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13. Yang CY, Tsai SS, Cheng BH et al. Sex Ratio at Birth Associated with Petrochemical Air Pollution in Taiwan. *Bulletin of Environmental Contamination and Toxicology* 2000,65(1): 126-131.

14. Davis DL, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA : the journal of the American Medical Association* 1998,279(13): 1018-1023.

15. Watanabe N, Kurita M. The Masculinization of the Fetus During Pregnancy Due to Inhalation of Diesel Exhaust. *Environmental health perspectives* 2001,109(2).

16. Lichtenfels AJ, Gomes JB, Pieri PC et al. Increased levels of air pollution and a decrease in the human and mouse male-to-female ratio in Sao Paulo, Brazil. *Fertil Steril* 2007,87(1): 230-232.

17. Fundação Seade. http://www.seade.gov.br/produtos/pib/index.php Acessed on 08/07/2012.

18. CETESB. Qualidade do ar no estado de São Paulo 2011. São Paulo : CETESB, 2012.

19. Williams FL, Lawson AB, Lloyd OL. Low sex ratios of births in areas at risk from air pollution from incinerators, as shown by geographical analysis and 3-dimensional mapping. Int J Epidemiol. 1992 ;21(2):311-9.

20. Lloyd OL, Smith G, Lloyd MM et al.. Raised mortality from lung cancer and high sex ratios of births associated with industrial pollution. Br J Ind Med. 1985 ;42(7):475-80.

21. James WH. Hypotheses on the stability and variation of human sex ratios at birth. J Theor Biol. 2012;310:183-6. doi: 10.1016/j.jtbi.2012.06.038. Epub 2012 Jul 7.

22. James WH. The categories of evidence relating to the hypothesis that mammalian sex ratios at birth are causally related to the hormone concentrations of both parents around the time of conception. J Biosoc Sci. 2011;43(2):167-84.

23. Sánchez-Ccoyllo OR, Ynoue RY, Martins LD et al.. Vehicular particulate matter emissions in road tunnels in São Paulo, Brazil. Environ Monit Assess 2009; 149:241-9.

24. Carvalho-Oliveira A, Pozo RMK, Lobo DJA et al. Diesel emissions significantly influence composition and mutagenicity of ambient particles: a case study in São Paulo, Brazil. Environ Res 2005; 98:1-7.

25. Mattison, D.R.,Thomford,P.J.,1989.The mechanisms of action of reproductive toxicants.Toxicol.Pathol.17,364–376.

26. Borman, S.M., Christian,P.J.,Sipes,I.G. et al.Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol.Appl.Pharmacol.2000 - 167,191–198.

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27. Waldron I. Recent trends in sex mortality ratios for adults in developed countries. *Soc Sci Med* 1993,36(4): 451-462.

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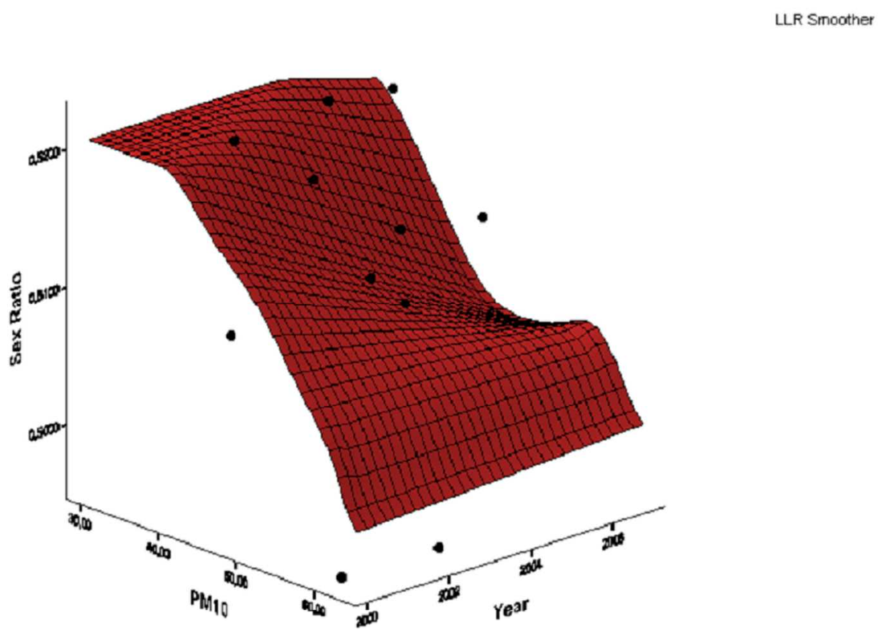

Fig. 1. Relation between Sex Ratio and PM10 in the period (2000-2007)
1297x1058mm (96 x 96 DPI)

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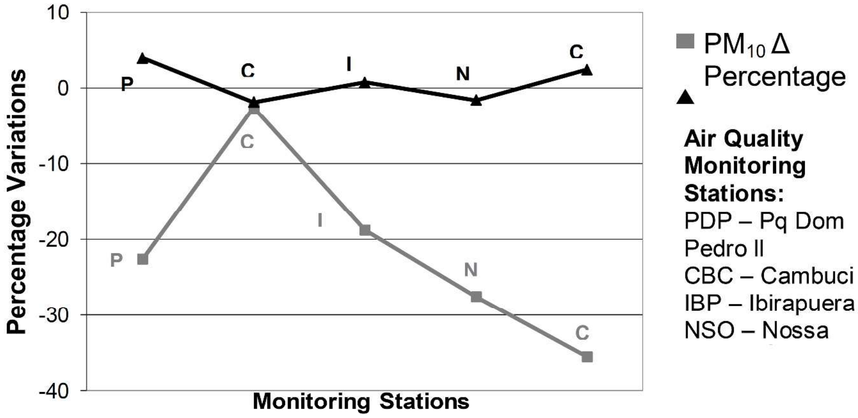

Fig. 2. PM10 and SSR percentage variations in the period (2000-2007) for the different monitoring station.
1155x670mm (96 x 96 DPI)

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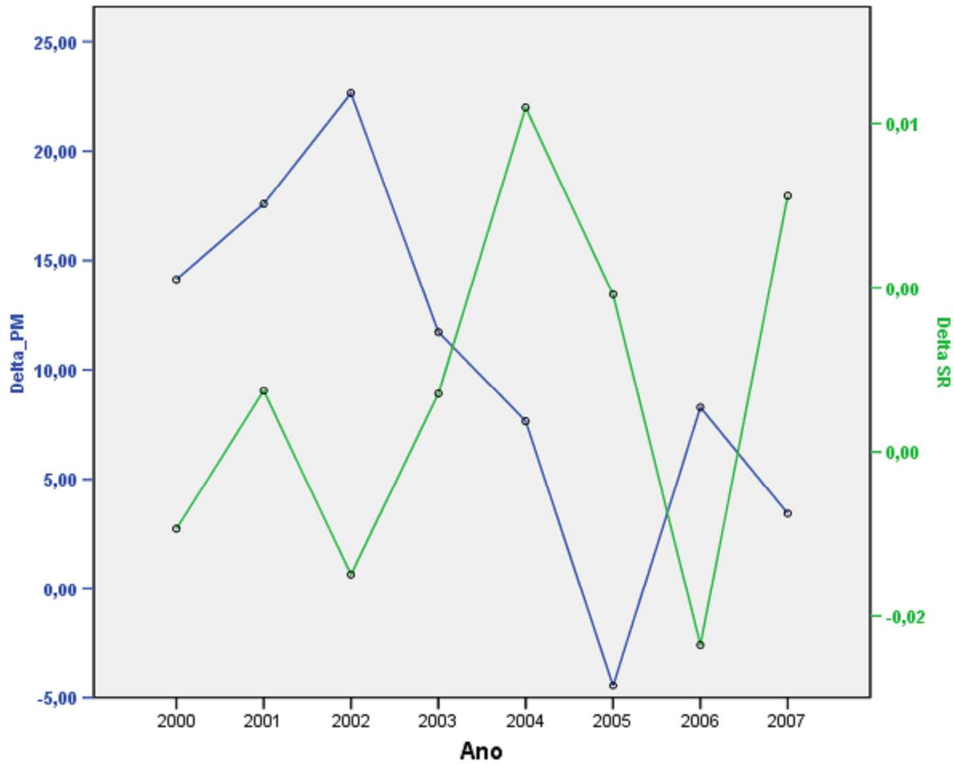

Fig. 3. Delta PM10 and delta SSR percentage variations along the analyzed period (2000-2007)
1322x1058mm (96 x 96 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *times series studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Non applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	Non applicable
Study size	10	Explain how the study size was arrived at	Non applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Non applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	Non applicable
		(*c*) Explain how missing data were addressed	Non applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Non applicable
		(*e*) Describe any sensitivity analyses	Non applicable
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Non applicable
		(b) Give reasons for non-participation at each stage	Non applicable
		(c) Consider use of a flow diagram	Non applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Non applicable
		(b) Indicate number of participants with missing data for each variable of interest	Non applicable
Outcome data	15*	Report numbers of outcome events or summary measures	Non applicable
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	9-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Non applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Non applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Non applicable

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil - a times series study.

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Keywords: Sex ratio, air pollution, reproductive health, environmental health, São Paulo

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ABSTRACT

Background SSR (Secondary Sex Ratio) has become an indicator of population balance. Scarce studies have found a direct association of environmental pollution and changes in SSR.

Objectives In order to assess if ambient air pollution in urban areas could be related to alterations in male/female proportion this study objectives to evaluate changes in ambient particulate matter (PM_{10}) concentrations after implementation of pollution control programs in São Paulo city and the secondary sex ratio (SRR).

Design and Methods A times series study was conducted. São Paulo's districts were stratified according to the PM_{10} concentrations levels and were used as a marker of overall air pollution. The male proportion was chosen to represent the secondary sex ratio (SSR=total male birth/total births). The SSR data from each area was analyzed according to the time variation and PM_{10} concentration' areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

Results The exponential regression showed a negative and significant association between PM_{10} and SSR. SSR varied from 51.4% to 50.7 % in São Paulo in the analyzed period (2000-2007). Considering the PM_{10} average concentration in São Paulo city of 44.72 $\mu g/m^3$ in the study period, the SSR decline reached almost 4.37%, equivalent to 30,934 less male births

Conclusion Ambient levels of PM_{10} are negatively associated with changes in the SSR. Therefore, we can speculate that higher levels of particulate pollution could be related to increased rates of female births.

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Article summary

1) Article Focus

- Study the potential influence of air pollution in gender in Sao Paulo in an extended time series period
- Discuss the future impacts of imbalance gender proportionality in urban centers

2) Key Messages

- Air pollution may influence gender determination
- Scarce studies showing this effect in urban centers
- Higher levels of air pollution may be associated to the increase rates of female births

3) Strengths and Limitations.

- We analyzed male/female births in different areas of São Paulo, Brazil.
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- SSR varied from 51.4% to 50.7 %, suggesting that air pollution may be associated to changes

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Summary Box

What is already known on this subject?

Air pollution is an environmental risk factor of concern in urban centers all over the world. Reductions in secondary sex ratio has been suggested to be indicative of potential influences of polluted environments on reproductive function. Previous study analyzed the relationship between air pollution and secondary sex ratio in an urban center of a developing country in a restricted time series period.

What does this study add?

The pollution levels in the city has declined significantly due to the national pollution control program since previous evaluation; thus this study extended the period analysis in order to assess if changes in the particulate matter concentration are followed by changes in secondary sex ratio. Data have shown a strong association and could indicate SSR as a potential indicator of population health status orientating future public policies for environmental control.

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Contributorship Statement

The corresponding author has conceived and planned the study design and analysis; all the authors have contributed with the analysis, elaboration and final approval of the manuscript.

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INTRODUCTION

Air pollution is an environmental risk factor of concern in urban centers all over the world. Respiratory and cardiovascular diseases are the most commonly observed and associated effects followed by neoplasia,[1, 2, 3]. However, in the last two decades lesser known effects associated to chronic air pollution exposures have started to emerge [4]. New epidemiological and experimental studies link exposure to reproductive adverse outcomes and investigations have risen different effects to be attributed to air pollution such as low birth weight,[5] miscarriages, [6], preterm birth [7] and decrease sperm quality [8].

Secondary sex ratio (number of male births in relation to total births) seems to be affected in population living in polluted environments and occupationally exposed to certain chemicals,[9,10,11,12,13]. Although the causality between environmental exposures and declines in secondary sex ration are still controversial, some authors suggest that the SSR as a sentinel indicator of reproductive injury and avoidable health exposures,[14] due to environmental pollution.

Experimental evidence indicate that prenatal exposure to air pollution derived from diesel exhausts is associated with altered sexual differentiation and function,[15]. Studies in humans and animals have found a reduction in the number of male births associated with lower male fertility, but the mechanism by which environmental hazards might change the sex ratio has not yet been established [9,16].

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In a previous study, we have demonstrated a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}),[16]. This study was conducted in São Paulo Metropolitan Region (SPMR) in Brazil, and the area was divided in terms of level of PM_{10} concentrations. Findings indicated a SSR of 51.7% for the less polluted area whereas for highly polluted area the proportion decreased to 50.7%. This result corresponds to a difference of 1% in total male births, or 1,180 fewer male births in the most polluted regions),[16].

Previous data analyzed a restricted time series period (2001-2003) and during the last years air pollution levels in the city has changed significantly due to the national pollution control program (PROCONVE and PROMOT). In this sense, it is desirable to verify if changes in levels of air pollution are accompanied by concurrent changes in the SSR in SPMR. Thus, the purpose of this study was to extend the time period evaluating from 2000 to 2007 to assess changes in ambient particulate matter (PM_{10}) concentrations and secondary sex ratio in the RMSP during this period (SRR).

METHODS

Number of births according to gender

The total number of live births in São Paulo was collected from 2000 to 2007 in a monthly basis representing a sample of 53,612 births. These records were obtained from SEADE, a public foundation which registers population data in the State of São Paulo. The male proportion was chosen to represent the secondary sex ratio (SSR=total male birth/total births).

Studied Area

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São Paulo is the largest Brazilian city, where most important economic activity is concentrated and is responsible for 17% of the country's gross national product. São Paulo is considered the 6th largest city in the world with a population of approximately 11 million in an area of 7,943.82 km² [17].

According to the São Paulo's Environmental State Agency air pollution is derived mostly by vehicles (combustion and re-suspension) and a small industrial contribution. Winter period in São Paulo favors thermal inversions and this may also contribute to non-favorable pollutant dispersion scenario and increased levels of PM_{10} [18]. Air pollution control programs in São Paulo Metropolitan Area are well succeed for the fixed sources however the mobile sources are of government concern.

Air pollution Data

The studied area encompasses the sub-districts where the state environmental agency (CETESB) has air monitoring stations, and were selected according to different air pollution gradients (high and low concentrations' areas). In the case of São Paulo we have a reasonable well spread air pollution monitoring system so we assumed that concentrations of a given region reflect somehow exposure. Since we have information about the birth outcomes aggregated by administrative districts in São Paulo, we assumed that the station located in a given districted would reasonably reflect exposure of pregnant women living in the given district. We couldn't reach information on the mothers' mobility during pregnancy or other mothers' data. We considered that pollution affects the mothers independently. We included districts for which we had a good quality representative data (valid time series) and stratified according to the PM_{10} levels. The districts were aggregated according to the level of PM_{10} concentration as

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follow: high level (≥40 μg/m^3) and low level (< 40 μg/m^3). PM_{10} concentrations were used as a marker of overall air pollution.

In total data were obtained from 5 automatic monitoring stations maintained by CETESB. In all stations, PM_{10} was measured through inter compared beta radiation monitors. The daily values obtained from each station were averaged in a monthly basis and considered as indicative of city-wide pollution levels. There is a correlation between PM_{10} concentrations registered at the different sites that means that PM_{10} is regularly distributed along the citywide.

Statistical analysis

The SSR data from each area were analyzed according to the time variation and PM_{10} concentration in the areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

RESULTS

The sub district's average concentrations of PM_{10} in the period ranged from 34.1 μg/m^3 to 64μg/m^3 and the SSR from 0.49 to 0.52 as depicted in Fig. 1.

In the less polluted area, the SSR average was 51.4% for 28,022 births recorded whereas in the most polluted area the proportion decreased to 50.7 % for 22,590 births recorded. We observed a general decrease trend in PM_{10} concentrations through the analyzed time period while the SSR simultaneously presented an increase.

An analysis of percentage variations considering the extreme years of the time-series analysis (that is, 2007 compared to 2001) was conducted showing a continuous decrease of PM_{10} concentration associated to an increase in SSR in each monitoring

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sub-district in the period, except for one monitoring station, which presented the same average level (Fig. 2). Surprisingly, Cambuci (CBC) monitoring station presented no variation in both variables (PM_{10} and SSR); however, this finding confirms the association observed in the other stations where lower PM_{10} concentrations are related to higher SSR.

The exponential regression showed a negative and significant association between PM_{10} and SSR (Table 1).

Table 1. Bivariate exponential regression analysis and relative risk.

Variable	R^2	β	p-value	RR
SSR	0,322	-0.001	0.022	0,999

SSR: Secundary Sex Ratio; RR: Relative Risk

Fig. 3 emphasizes the inversely relationship of PM_{10} concentrations and SSR, specially from 2002 on, when we can observe the annual variations in both variables occurring in opposite directions, reinforcing the above demonstrated findings.

DISCUSSION

In this study we have evaluated the variation in PM_{10} environmental concentration and SSR in the Metropolitan Region of Sao Paulo, Brazil during the years of 2001-2007. In a previous study conducted in the same area we have noted that there was a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10})[16]. In this study we extended analyzed time period, which allowed us to observe improvements in air quality due to the environmental control politics introduced (motorized vehicles' emissions control) and in the

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population's gender pattern. Although the air quality increased we still find a significant negative association between the SSR and PM_{10} concentration.

Based on CETESB (São Paulo Environmental State Agency) report [18] and several studies conducted in São Paulo [19, 20], the receptor models using chemical comprehensive characterization of particles indicated that 90% of PM10 is generated by vehicles or photochemical process generated by traffic. PM10 is not a single pollutant; is a synthesis of carbon and varies its composition; it carries primary and secondary pollutants. In the referred stations there were an improvement of the diesel fuel and motors' technology, added by a traffic detour due to an infrastructure road implementation (this behaviour was observed in PDP station).

In one region of the city, where CBC station is located (central area of São Paulo city) no variation in PM_{10} was noted and we can speculate that this no variation in PM_{10} is due by the fact that this area has buses emissions as main air pollution source, with lower contributions from cars and motorcycles. No variations in PM_{10} in this region shows that air pollution control program have not positively impacted the area leading to the maintenance of the air pollution level. Maintenance of PM_{10} levels was accompanied by maintenance of the SSR for this region. CBC station records and associated SSR can be interpreted as a control unit for other stations where there were variations in PM_{10} concentration meaning that for the same level of air pollution the same SSR was registered.

These results could suggest that there is a possible contribution of PM_{10} levels in SSR variation, explaining more than 30% of the events. If we consider that there is causal relationship the increase of 10µg/m³ in PM_{10} concentration would lead to a decline of 0.995% in SSR. Further, taking into account a PM_{10} average concentration in

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São Paulo city of 44.72 μg/m^3 in this study period the SSR decline would reach almost 4.37% which is equivalent to 30,934 less male births.

This behavior (decrease in PM_{10} and increase in SSR) is consistent with previous findings ,[15] that have shown a possible association between exposure to urban air pollution and imbalance of the sex ratio at birth. Other studies have also reported lower sex ratio in residential areas at risk from air pollution emitted from incinerators [21] as well as higher sex ratio in areas exposed to polluted air from steel foundry [22].

In humans the sex of the baby is determined primarily by the fecundation of the X egg by the X (female) or Y (male) sperm. In the case of environmental exposures and changes in the secondary sex ratio as a health outcome, it is very difficult to determine the time connection between sex at birth because the effect could have occurred before, during pregnancy. Further, changes in the sex ratio may be associated with maternal or paternal factors or with both. Pre implantation hypothesis propose that in some circumstances there are more favorable development or survival of X or Y bearing sperm or survival of male or female embryos [23-25]. In a previous study of our group we have shown that exposure to PM during the preconception period are associated to early pregnancy loss in women undergoing in vitro fertilization [26] and thus there is also another the possibility to explain the changes in sex ratio by sex specific increases in intrauterine death or stillbirth.

Potential toxicological mechanisms that might explain and give strength to the environmental contamination causes in the determination of the sex ratio are still inconclusive. There are some suggestions in the literature that include the hormonal status of the parents at the time of conception, differential characteristics and sensibility of sperm of one sex, combination and presence of specific toxic substances (PAH, dioxin) [27,28]. Although we have not evaluated the elemental composition of PM10,

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previous studies have characterized the composition of these particles from São Paulo city. Chemical elements included Fe, Br, Al, Si, S, Cu, Zn, Pb [29] and PAH such as benzene, toluene, etil-benzene e xylene [30]. Toxicological studies have shown that certain toxicants present in ambient air pollution, such as PAH and heavy metals potential endocrine disruptors [31,32].

This is a descriptive study which does not intend to implicate in causality and it subsidies on a previous research [16]. The changes in air pollution were compatible with the effects' variation and there is a toxicological support for that [16]. In this sense, it is a limitation but once it is a trend study and the measures to be aggregated are monthly records (SSR) and daily measures (PM10), a synchrony between exposure and gender determination is minimized when you aggregate data on yearly basis. This is a situation different from a classical times series study because you know exactly the time relationship between exposure and health outcome (death or hospital admission). In the case of considering SSR as a health outcome, it is very difficult to determine the time connection between exposure and sex at birth, as previously demonstrated it can occur pre-fecundation, implantation or in the gestation. When you aggregate the data in a yearly basis you encompass these phases, therefore in a times series study we can not capture this effect. It could be done in a birth cohort study but once these prematurity are scarce events the size of the sample would become this complex and costly study.

Increasing differences in the proportion of male/female births can lead, in a mid-long term future, to a deficit in male's population and cause social problems. This scenario gets worst if we consider that male are more prone to premature death because of their trend to engage in risk behavior and violence,[33].

The air pollution control programs (PROCONVE and PROMOT, which refers to emissions limit to new motor vehicles – cars and motorbikes) may have contributed to

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the improvement in the air quality parameters registered through the decade. Recently, an inspection and maintenance program concerning emissions limits for the old and second handed vehicles was implemented in São Paulo and that may also have favored this scenario. Our findings are important indicators for an advance of the public health endpoints due to the improvement of the air quality in urban centers. Considering the disproportion in the male/female births, this balance is desirable to achieve and maintain in all populations of urban centers. Furthermore, the abatement of air pollution is a target that governments must pursue.

CONCLUSIONS

Although the biological mechanisms responsible for the SSR changes are not clearly established, this study indicate that concentration of particulate air pollution in urban cities are associated with decreased SSR. Also, this data give support for the use of SSR as a potential indicator of the negative health impacts of fuels combustion derived emissions in urban cities.

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REFERENCES

1. Schwartz J.. Long-term effects of exposure to particulate air pollution. *Clin Occup Environ Med* 2006,5(4):837-848.

2. Pope CA, 3rd, Ezzati M, Dockery DW.. Fine-particulate air pollution and life expectancy in the United States. *The New England journal of medicine* 2009,360(4): 376-386.

3. Dockery DW. Health effects of particulate air pollution. *Ann Epidemiol* 2009,19(4): 257-263.

4. Calderon-Garciduenas L, Engle R, Mora-Tiscareno A et al. Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and cognition* 2011,77(3): 345-355.

5. Gouveia N, Bremner SA, Novaes HMD. Association between ambient air pollution and birth weight in São Paulo, Brazil. *Journal of Epidemiology and Community Health* 2004,58(1): 11-17.

6. Mohorovic L, Petrovic O, Haller H et al. Pregnancy loss and maternal methemoglobin levels: an indirect explanation of the association of environmental toxics and their adverse effects on the mother and the fetus. Int J Environ Res Public Health.2010 Dec;7(12):4203-12. Epub 2010.

7. van den Hooven EH, Pierik FH, de Kluizenaar Y et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environmental health perspectives* 2012,120(1): 150-156.

8. Pires A, de Melo EN, Mauad T et al. Pre- and postnatal exposure to ambient levels of urban particulate matter (PM(2.5)) affects mice spermatogenesis. *Inhal Toxicol* 2011,23(4): 237-245.

9. Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. *Emerging Health Threats Journal* 2011;Vol 4 (2011) incl Supplements.

10. Tragaki A, Lasaridi K. Temporal and spatial trends in the sex ratio at birth in Greece, 1960–2006: exploring potential environmental factors. *Population & Environment* 2009,30(3): 114-128.

11. Schnorr TM, Lawson CC, Whelan EA et al. Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environmental health perspectives* 2001,109(11): 1127-1132.

12. Ryan JJ, Amirova Z, Carrier G.Sex ratios of children of Russian pesticideproducers exposed to dioxin. EnvironHealth Perspect. 2002;110(11):A699-701.

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13. Yang CY, Tsai SS, Cheng BH et al. Sex Ratio at Birth Associated with Petrochemical Air Pollution in Taiwan. *Bulletin of Environmental Contamination and Toxicology* 2000,65(1): 126-131.

14. Davis DL, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA : the journal of the American Medical Association* 1998,279(13): 1018-1023.

15. Watanabe N, Kurita M. The Masculinization of the Fetus During Pregnancy Due to Inhalation of Diesel Exhaust. *Environmental health perspectives* 2001,109(2).

16. Lichtenfels AJ, Gomes JB, Pieri PC et al. Increased levels of air pollution and a decrease in the human and mouse male-to-female ratio in Sao Paulo, Brazil. *Fertil Steril* 2007,87(1): 230-232.

17. Fundação Seade. http://www.seade.gov.br/produtos/pib/index.php Acessed on 08/07/2012.

18. CETESB. Qualidade do ar no estado de São Paulo 2011. São Paulo : CETESB, 2012.

19. Miranda, R. M**.** ; Andrade, M. F. ; Fornaro, A. ; et al. Urban air pollution: a representative survey of PM2.5 mass concentrations in six Brazilian cities. Air quality Atm and Health, v. 5, p. 63, 2012.

20. Andrade, M. F. ; Miranda, R. M**.** ; Fornaro, A. et al. Vehicle emissions and PM2.5 mass concentrations in six Brazilian cities. Air quality atmosphere and health, v. 5, p. 79, 2012.

21. Williams FL, Lawson AB, Lloyd OL. Low sex ratios of births in areas at risk from air pollution from incinerators, as shown by geographical analysis and 3-dimensional mapping. Int J Epidemiol. 1992 ;21(2):311-9.

22. Lloyd OL, Smith G, Lloyd MM et al.. Raised mortality from lung cancer and high sex ratios of births associated with industrial pollution. Br J Ind Med. 1985 ;42(7):475-80.

23. Mocarelli P, Gerthoux PM, Patterson DG Jr, et al. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ Health Perspect. 2008;116(1):70-7.

24. Hansen D, Moller H and Olsen J Severe periconceptional life events and the sex ratio in offspring: follow up study based on five national registers. Br Med J 1999; 319:548–549.

25. Boklage CE.The epigenetic environment: secondary sex ratio depends on differential survival in embryogenesis. Hum Reprod. 2005 Mar;20(3):583-7.

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26. Perin PM, Maluf M, Czeresnia CE, et al. . Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). J Assist Reprod Genet. 2010; 27(7):371-82.

27. James WH. Hypotheses on the stability and variation of human sex ratios at birth. J Theor Biol. 2012;310:183-6. doi: 10.1016/j.jtbi.2012.06.038. Epub 2012 Jul 7.

28. James WH. The categories of evidence relating to the hypothesis that mammalian sex ratios at birth are causally related to the hormone concentrations of both parents around the time of conception. J Biosoc Sci. 2011;43(2):167-84.

29. Sánchez-Ccoyllo OR, Ynoue RY, Martins LD et al.. Vehicular particulate matter emissions in road tunnels in São Paulo, Brazil. Environ Monit Assess 2009; 149:241-9.

30. Carvalho-Oliveira A, Pozo RMK, Lobo DJA et al. Diesel emissions significantly influence composition and mutagenicity of ambient particles: a case study in São Paulo, Brazil. Environ Res 2005; 98:1-7.

31. Mattison, D.R.,Thomford,P.J.,1989.The mechanisms of action of reproductive toxicants.Toxicol.Pathol.17,364–376.

32. Borman, S.M., Christian,P.J.,Sipes,I.G. et al.Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol.Appl.Pharmacol.2000 - 167,191–198.

33. Waldron I. Recent trends in sex mortality ratios for adults in developed countries. *Soc Sci Med* 1993,36(4): 451-462.

LIST OF FIGURES

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Air pollution is an environmental risk factor of concern in urban centers all over the world. Reductions in secondary sex ratio has been suggested to be indicative of potential influences of polluted environments on reproductive function. Previous study analyzed the relationship between air pollution and secondary sex ratio in an urban center of a developing country in a restricted time series period.

What does this study add?

The pollution levels in the city has declined significantly due to the national pollution control program since previous evaluation; thus this study extended the period analysis in order to assess if changes in the particulate matter concentration are followed by changes in secondary sex ratio. Data have shown a strong association and could indicate SSR as a potential indicator of population health status orientating future public policies for environmental control.

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Contributorship Statement

The corresponding author has conceived and planned the study design and analysis; all the authors have contributed with the analysis, elaboration and final approval of the manuscript.

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INTRODUCTION

Air pollution is an environmental risk factor of concern in urban centers all over the world. Respiratory and cardiovascular diseases are the most commonly observed and associated effects followed by neoplasia,[1, 2, 3]. However, in the last two decades lesser known effects associated to chronic air pollution exposures have started to emerge [4]. New epidemiological and experimental studies link exposure to reproductive adverse outcomes and investigations have risen different effects to be attributed to air pollution such as low birth weight,[5] miscarriages, [6], preterm birth [7] and decrease sperm quality [8].

Secondary sex ratio (number of male births in relation to total births) seems to be affected in population living in polluted environments and occupationally exposed to certain chemicals,[9,10,11,12,13]. Although the causality between environmental exposures and declines in secondary sex ration are still controversial, some authors suggest that the SSR as a sentinel indicator of reproductive injury and avoidable health exposures,[14] due to environmental pollution.

Experimental evidence indicate that prenatal exposure to air pollution derived from diesel exhausts is associated with altered sexual differentiation and function,[15]. Studies in humans and animals have found a reduction in the number of male births associated with lower male fertility, but the mechanism by which environmental hazards might change the sex ratio has not yet been established [9,16].

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In a previous study, we have demonstrated a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}), [16]. This study was conducted in São Paulo Metropolitan Region (SPMR) in Brazil, and the area was divided in terms of level of PM_{10} concentrations. Findings indicated a SSR of 51.7% for the less polluted area whereas for highly polluted area the proportion decreased to 50.7%. This result corresponds to a difference of 1% in total male births, or 1,180 fewer male births in the most polluted regions),[16].

Previous data analyzed a restricted time series period (2001-2003) and during the last years air pollution levels in the city has changed significantly due to the national pollution control program (PROCONVE and PROMOT). In this sense, it is desirable to verify if changes in levels of air pollution are accompanied by concurrent changes in the SSR in SPMR. Thus, the purpose of this study was to extend the time period evaluating from 2000 to 2007 to assess changes in ambient particulate matter (PM_{10}) concentrations and secondary sex ratio in the RMSP during this period (SRR).

METHODS

Number of births according to gender

The total number of live births in São Paulo was collected from 2000 to 2007 in a monthly basis representing a sample of 53,612 births. These records were obtained from SEADE, a public foundation which registers population data in the State of São Paulo. The male proportion was chosen to represent the secondary sex ratio (SSR=total male birth/total births).

Studied Area

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São Paulo is the largest Brazilian city, where most important economic activity is concentrated and is responsible for 17% of the country's gross national product. São Paulo is considered the 6th largest city in the world with a population of approximately 11 million in an area of 7,943.82 km² [17].

According to the São Paulo's Environmental State Agency air pollution is derived mostly by vehicles (combustion and re-suspension) and a small industrial contribution. Winter period in São Paulo favors thermal inversions and this may also contribute to non-favorable pollutant dispersion scenario and increased levels of PM_{10} [18]. Air pollution control programs in São Paulo Metropolitan Area are well succeed for the fixed sources however the mobile sources are of government concern.

Air pollution Data

The studied area encompasses the sub-districts where the state environmental agency (CETESB) has air monitoring stations, and were selected according to different air pollution gradients (high and low concentrations' areas). In the case of São Paulo we have a reasonable well spread air pollution monitoring system so we assumed that concentrations of a given region reflect somehow exposure. Since we have information about the birth outcomes aggregated by administrative districts in São Paulo, we assumed that the station located in a given districted would reasonably reflect exposure of pregnant women living in the given district. We couldn't reach information on the mothers' mobility during pregnancy or other mothers' data. We considered that pollution affects the mothers independently. We included districts for which we had a good quality representative data (valid time series) and stratified according to the PM_{10} levels. The districts were aggregated according to the level of PM_{10} concentration as

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follow: high level (≥40 μg/m^3) and low level (< 40 μg/m^3). PM_{10} concentrations were used as a marker of overall air pollution.

In total data were obtained from 5 automatic monitoring stations maintained by CETESB. In all stations, PM_{10} was measured through inter compared beta radiation monitors. The daily values obtained from each station were averaged in a monthly basis and considered as indicative of city-wide pollution levels. There is a correlation between PM_{10} concentrations registered at the different sites that means that PM_{10} is regularly distributed along the citywide.

Statistical analysis

The SSR data from each area were analyzed according to the time variation and PM_{10} concentration in the areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

RESULTS

The sub district's average concentrations of PM_{10} in the period ranged from 34.1 μg/m^3 to 64μg/m^3 and the SSR from 0.49 to 0.52 as depicted in Fig. 1.

In the less polluted area, the SSR average was 51.4% for 28,022 births recorded whereas in the most polluted area the proportion decreased to 50.7 % for 22,590 births recorded. We observed a general decrease trend in PM_{10} concentrations through the analyzed time period while the SSR simultaneously presented an increase.

An analysis of percentage variations considering the extreme years of the time-series analysis (that is, 2007 compared to 2001) was conducted showing a continuous decrease of PM_{10} concentration associated to an increase in SSR in each monitoring

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sub-district in the period, except for one monitoring station, which presented the same average level (Fig. 2). Surprisingly, Cambuci (CBC) monitoring station presented no variation in both variables (PM_{10} and SSR); however, this finding confirms the association observed in the other stations where lower PM_{10} concentrations are related to higher SSR.

The exponential regression showed a negative and significant association between PM_{10} and SSR (Table 1).

Table 1. Bivariate exponential regression analysis and relative risk.

Variable	R^2	β	p-value	RR
SSR	0,322	-0.001	0.022	0,999

SSR: Secundary Sex Ratio; RR: Relative Risk

Fig. 3 emphasizes the inversely relationship of PM_{10} concentrations and SSR, specially from 2002 on, when we can observe the annual variations in both variables occurring in opposite directions, reinforcing the above demonstrated findings.

DISCUSSION

In this study we have evaluated the variation in PM_{10} environmental concentration and SSR in the Metropolitan Region of Sao Paulo, Brazil during the years of 2001-2007. In a previous study conducted in the same area we have noted that there was a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10})[16]. In this study we extended analyzed time period, which allowed us to observe improvements in air quality due to the environmental control politics introduced (motorized vehicles' emissions control) and in the

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population's gender pattern. Although the air quality increased we still find a significant negative association between the SSR and PM_{10} concentration.

Based on CETESB (São Paulo Environmental State Agency) report [18] and several studies conducted in São Paulo [19, 20], the receptor models using chemical comprehensive characterization of particles indicated that 90% of PM10 is generated by vehicles or photochemical process generated by traffic. PM10 is not a single pollutant; is a synthesis of carbon and varies its composition; it carries primary and secondary pollutants. In the referred stations there were an improvement of the diesel fuel and motors' technology, added by a traffic detour due to an infrastructure road implementation (this behaviour was observed in PDP station).

In one region of the city, where CBC station is located (central area of São Paulo city) no variation in PM_{10} was noted and we can speculate that this no variation in PM_{10} is due by the fact that this area has buses emissions as main air pollution source, with lower contributions from cars and motorcycles. No variations in PM_{10} in this region shows that air pollution control program have not positively impacted the area leading to the maintenance of the air pollution level. Maintenance of PM_{10} levels was accompanied by maintenance of the SSR for this region. CBC station records and associated SSR can be interpreted as a control unit for other stations where there were variations in PM_{10} concentration meaning that for the same level of air pollution the same SSR was registered.

These results could suggest that there is a possible contribution of PM_{10} levels in SSR variation, explaining more than 30% of the events. If we consider that there is causal relationship the increase of 10µg/m³ in PM_{10} concentration would lead to a decline of 0.995% in SSR. Further, taking into account a PM_{10} average concentration in

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São Paulo city of 44.72 μg/m^3 in this study period the SSR decline would reach almost 4.37% which is equivalent to 30,934 less male births.

This behavior (decrease in PM_{10} and increase in SSR) is consistent with previous findings ,[15] that have shown a possible association between exposure to urban air pollution and imbalance of the sex ratio at birth. Other studies have also reported lower sex ratio in residential areas at risk from air pollution emitted from incinerators [21] as well as higher sex ratio in areas exposed to polluted air from steel foundry [22].

In humans the sex of the baby is determined primarily by the fecundation of the X egg by the X (female) or Y (male) sperm. In the case of environmental exposures and changes in the secondary sex ratio as a health outcome, it is very difficult to determine the time connection between sex at birth because the effect could have occurred before, during pregnancy. Further, changes in the sex ratio may be associated with maternal or paternal factors or with both. Pre implantation hypothesis propose that in some circumstances there are more favorable development or survival of X or Y bearing sperm or survival of male or female embryos [23-25]. In a previous study of our group we have shown that exposure to PM during the preconception period are associated to early pregnancy loss in women undergoing in vitro fertilization [26] and thus there is also another the possibility to explain the changes in sex ratio by sex specific increases in intrauterine death or stillbirth.

Potential toxicological mechanisms that might explain and give strength to the environmental contamination causes in the determination of the sex ratio are still inconclusive. There are some suggestions in the literature that include the hormonal status of the parents at the time of conception, differential characteristics and sensibility of sperm of one sex, combination and presence of specific toxic substances (PAH, dioxin) [27,28]. Although we have not evaluated the elemental composition of PM10,

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previous studies have characterized the composition of these particles from São Paulo city. Chemical elements included Fe, Br, Al, Si, S, Cu, Zn, Pb [29] and PAH such as benzene, toluene, etil-benzene e xylene [30]. Toxicological studies have shown that certain toxicants present in ambient air pollution, such as PAH and heavy metals potential endocrine disruptors [31,32].

This is a descriptive study which does not intend to implicate in causality and it subsidies on a previous research [16]. The changes in air pollution were compatible with the effects' variation and there is a toxicological support for that [16]. In this sense, it is a limitation but once it is a trend study and the measures to be aggregated are monthly records (SSR) and daily measures (PM10), a synchrony between exposure and gender determination is minimized when you aggregate data on yearly basis. This is a situation different from a classical times series study because you know exactly the time relationship between exposure and health outcome (death or hospital admission). In the case of considering SSR as a health outcome, it is very difficult to determine the time connection between exposure and sex at birth, as previously demonstrated it can occur pre-fecundation, implantation or in the gestation. When you aggregate the data in a yearly basis you encompass these phases, therefore in a times series study we can not capture this effect. It could be done in a birth cohort study but once these prematurity are scarce events the size of the sample would become this complex and costly study.

Increasing differences in the proportion of male/female births can lead, in a mid-long term future, to a deficit in male's population and cause social problems. This scenario gets worst if we consider that male are more prone to premature death because of their trend to engage in risk behavior and violence,[33].

The air pollution control programs (PROCONVE and PROMOT, which refers to emissions limit to new motor vehicles – cars and motorbikes) may have contributed to

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the improvement in the air quality parameters registered through the decade. Recently, an inspection and maintenance program concerning emissions limits for the old and second handed vehicles was implemented in São Paulo and that may also have favored this scenario. Our findings are important indicators for an advance of the public health endpoints due to the improvement of the air quality in urban centers. Considering the disproportion in the male/female births, this balance is desirable to achieve and maintain in all populations of urban centers. Furthermore, the abatement of air pollution is a target that governments must pursue.

CONCLUSIONS

Although the biological mechanisms responsible for the SSR changes are not clearly established, this study indicate that concentration of particulate air pollution in urban cities are associated with decreased SSR. Also, this data give support for the use of SSR as a potential indicator of the negative health impacts of fuels combustion derived emissions in urban cities.

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REFERENCES

1. Schwartz J.. Long-term effects of exposure to particulate air pollution. *Clin Occup Environ Med* 2006,5(4):837-848.

2. Pope CA, 3rd, Ezzati M, Dockery DW.. Fine-particulate air pollution and life expectancy in the United States. *The New England journal of medicine* 2009,360(4): 376-386.

3. Dockery DW. Health effects of particulate air pollution. *Ann Epidemiol* 2009,19(4): 257-263.

4. Calderon-Garciduenas L, Engle R, Mora-Tiscareno A et al. Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and cognition* 2011,77(3): 345-355.

5. Gouveia N, Bremner SA, Novaes HMD. Association between ambient air pollution and birth weight in São Paulo, Brazil. *Journal of Epidemiology and Community Health* 2004,58(1): 11-17.

6. Mohorovic L, Petrovic O, Haller H et al. Pregnancy loss and maternal methemoglobin levels: an indirect explanation of the association of environmental toxics and their adverse effects on the mother and the fetus. Int J Environ Res Public Health.2010 Dec;7(12):4203-12. Epub 2010.

7. van den Hooven EH, Pierik FH, de Kluizenaar Y et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environmental health perspectives* 2012,120(1): 150-156.

8. Pires A, de Melo EN, Mauad T et al. Pre- and postnatal exposure to ambient levels of urban particulate matter (PM(2.5)) affects mice spermatogenesis. *Inhal Toxicol* 2011,23(4): 237-245.

9. Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. *Emerging Health Threats Journal* 2011;Vol 4 (2011) incl Supplements.

10. Tragaki A, Lasaridi K. Temporal and spatial trends in the sex ratio at birth in Greece, 1960–2006: exploring potential environmental factors. *Population & Environment* 2009,30(3): 114-128.

11. Schnorr TM, Lawson CC, Whelan EA et al. Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environmental health perspectives* 2001,109(11): 1127-1132.

12. Ryan JJ, Amirova Z, Carrier G.Sex ratios of children of Russian pesticideproducers exposed to dioxin. EnvironHealth Perspect. 2002;110(11):A699-701.

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13. Yang CY, Tsai SS, Cheng BH et al. Sex Ratio at Birth Associated with Petrochemical Air Pollution in Taiwan. *Bulletin of Environmental Contamination and Toxicology* 2000,65(1): 126-131.

14. Davis DL, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA : the journal of the American Medical Association* 1998,279(13): 1018-1023.

15. Watanabe N, Kurita M. The Masculinization of the Fetus During Pregnancy Due to Inhalation of Diesel Exhaust. *Environmental health perspectives* 2001,109(2).

16. Lichtenfels AJ, Gomes JB, Pieri PC et al. Increased levels of air pollution and a decrease in the human and mouse male-to-female ratio in Sao Paulo, Brazil. *Fertil Steril* 2007,87(1): 230-232.

17. Fundação Seade. http://www.seade.gov.br/produtos/pib/index.php Acessed on 08/07/2012.

18. CETESB. Qualidade do ar no estado de São Paulo 2011. São Paulo : CETESB, 2012.

19. Miranda, R. M**.** ; Andrade, M. F. ; Fornaro, A. ; et al. Urban air pollution: a representative survey of PM2.5 mass concentrations in six Brazilian cities. Air quality Atm and Health, v. 5, p. 63, 2012.

20. Andrade, M. F. ; Miranda, R. M**.** ; Fornaro, A. et al. Vehicle emissions and PM2.5 mass concentrations in six Brazilian cities. Air quality atmosphere and health, v. 5, p. 79, 2012.

21. Williams FL, Lawson AB, Lloyd OL. Low sex ratios of births in areas at risk from air pollution from incinerators, as shown by geographical analysis and 3-dimensional mapping. Int J Epidemiol. 1992 ;21(2):311-9.

22. Lloyd OL, Smith G, Lloyd MM et al.. Raised mortality from lung cancer and high sex ratios of births associated with industrial pollution. Br J Ind Med. 1985 ;42(7):475-80.

23. Mocarelli P, Gerthoux PM, Patterson DG Jr, et al. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ Health Perspect. 2008;116(1):70-7.

24. Hansen D, Moller H and Olsen J Severe periconceptional life events and the sex ratio in offspring: follow up study based on five national registers. Br Med J 1999; 319:548–549.

25. Boklage CE.The epigenetic environment: secondary sex ratio depends on differential survival in embryogenesis. Hum Reprod. 2005 Mar;20(3):583-7.

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26. Perin PM, Maluf M, Czeresnia CE, et al. . Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). J Assist Reprod Genet. 2010; 27(7):371-82.

27. James WH. Hypotheses on the stability and variation of human sex ratios at birth. J Theor Biol. 2012;310:183-6. doi: 10.1016/j.jtbi.2012.06.038. Epub 2012 Jul 7.

28. James WH. The categories of evidence relating to the hypothesis that mammalian sex ratios at birth are causally related to the hormone concentrations of both parents around the time of conception. J Biosoc Sci. 2011;43(2):167-84.

29. Sánchez-Ccoyllo OR, Ynoue RY, Martins LD et al.. Vehicular particulate matter emissions in road tunnels in São Paulo, Brazil. Environ Monit Assess 2009; 149:241-9.

30. Carvalho-Oliveira A, Pozo RMK, Lobo DJA et al. Diesel emissions significantly influence composition and mutagenicity of ambient particles: a case study in São Paulo, Brazil. Environ Res 2005; 98:1-7.

31. Mattison, D.R.,Thomford,P.J.,1989.The mechanisms of action of reproductive toxicants.Toxicol.Pathol.17,364–376.

32. Borman, S.M., Christian,P.J.,Sipes,I.G. et al.Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol.Appl.Pharmacol.2000 - 167,191–198.

33. Waldron I. Recent trends in sex mortality ratios for adults in developed countries. *Soc Sci Med* 1993,36(4): 451-462.

LIST OF FIGURES

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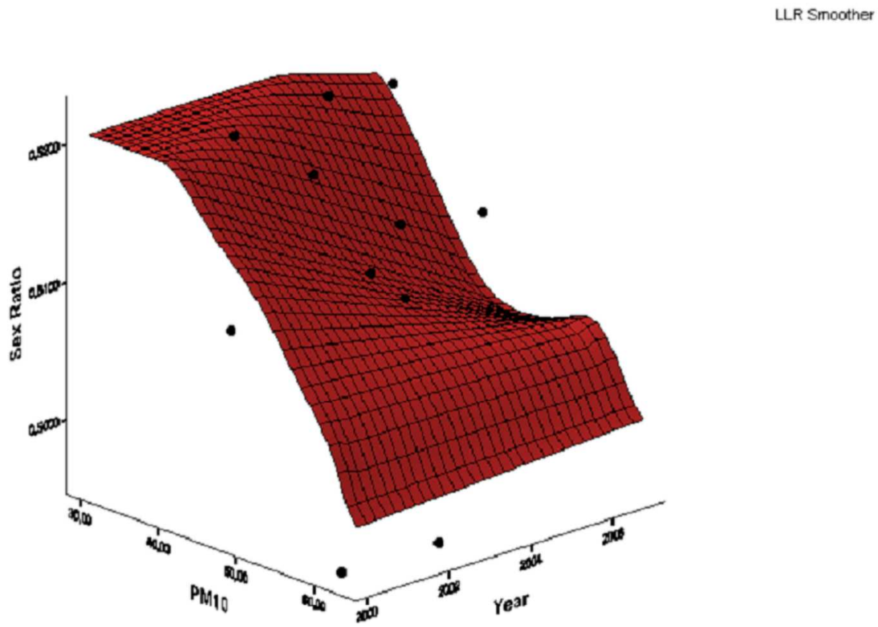

Fig. 1. Relation between Sex Ratio and PM10 in the period (2000-2007)
1297x1058mm (96 x 96 DPI)

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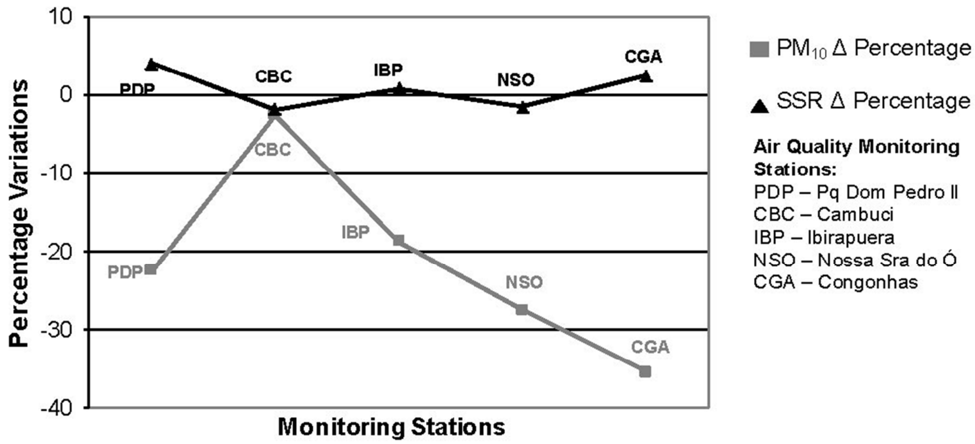

Fig. 2. PM10 and SSR percentage variations in the period (2000-2007) for the different monitoring station.
137x66mm (200 x 200 DPI)

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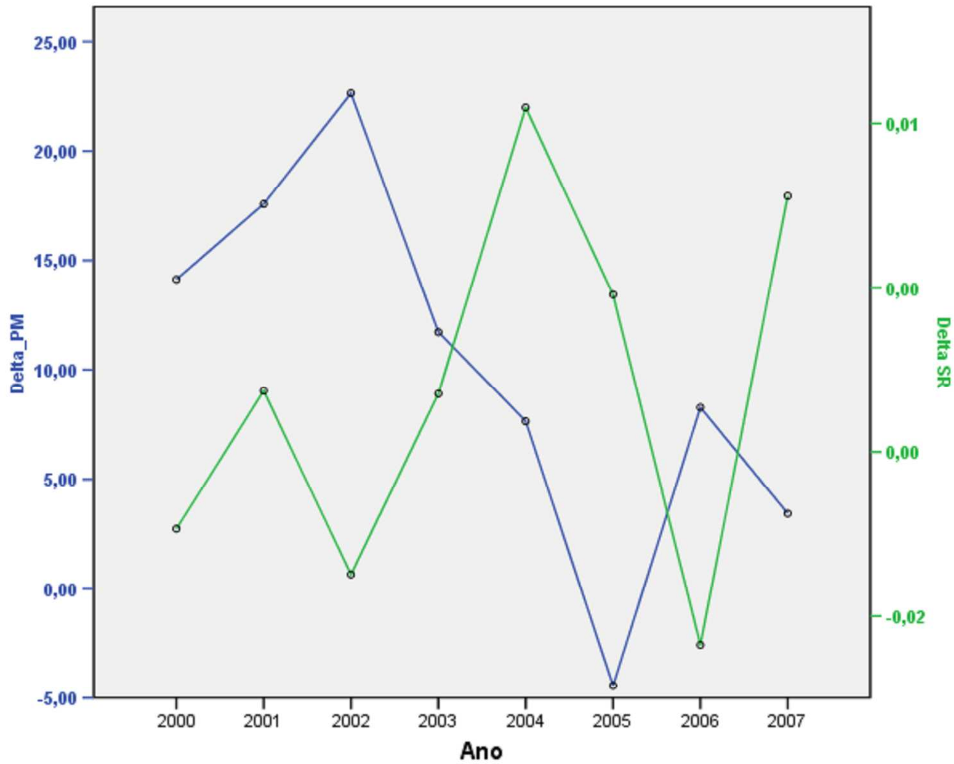

Fig. 3. Delta PM10 and delta SSR percentage variations along the analyzed period (2000-2007)
1322x1058mm (96 x 96 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *times series studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Non applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	Non applicable
Study size	10	Explain how the study size was arrived at	Non applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Non applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	Non applicable
		(*c*) Explain how missing data were addressed	Non applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Non applicable
		(*e*) Describe any sensitivity analyses	Non applicable
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Non applicable
		(b) Give reasons for non-participation at each stage	Non applicable
		(c) Consider use of a flow diagram	Non applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Non applicable
		(b) Indicate number of participants with missing data for each variable of interest	Non applicable
Outcome data	15*	Report numbers of outcome events or summary measures	Non applicable
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	9-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Non applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Non applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Non applicable

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil - a times series study

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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil - a times series study.

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Keywords: Sex ratio, air pollution, reproductive health, environmental health, São Paulo

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ABSTRACT

Background SSR (Secondary Sex Ratio) has become an indicator of population balance. Scarce studies have found a direct association of environmental pollution and changes in SSR.

Objectives In order to assess if ambient air pollution in urban areas could be related to alterations in male/female ratio this study objectives to evaluate changes in ambient particulate matter (PM_{10}) concentrations after implementation of pollution control programs in São Paulo city and the secondary sex ratio (SRR).

Design and Methods A time series study was conducted. São Paulo's districts were stratified according to the PM_{10} concentrations levels and were used as a marker of overall air pollution. The male ratio was chosen to represent the secondary sex ratio (SSR=total male birth/total births). The SSR data from each area was analyzed according to the time variation and PM_{10} concentration' areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

Results The exponential regression showed a negative and significant association between PM_{10} and SSR. SSR varied from 51.4% to 50.7 % in São Paulo in the analyzed period (2000-2007). Considering the PM_{10} average concentration in São Paulo city of 44.72 $\mu g/m^3$ in the study period, the SSR decline reached almost 4.37%, equivalent to 30,934 less male births

Conclusion Ambient levels of PM_{10} are negatively associated with changes in the SSR. Therefore, we can speculate that higher levels of particulate pollution could be related to increased rates of female births.

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Article summary

1) Article Focus

- Study the potential influence of air pollution in gender in Sao Paulo in an extended time series period
- Discuss the future impacts of imbalance gender proportionality in urban centers

2) Key Messages

- Air pollution may influence gender determination
- Scarce studies showing this effect in urban centers
- Higher levels of air pollution may be associated to the increase rates of female births

3) Strengths and Limitations.

- We analyzed male/female births in different areas of São Paulo, Brazil.
- We compared areas with different levels of PM_{10} concentration within the city
- The analysis period has a lag concerning exposure and outcome, once the sex definition occurs during the embryonic/conception period that could not be at the same year of birth.
- SSR varied from 51.4% to 50.7 %, suggesting that air pollution may be associated to changes

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Summary Box

What is already known on this subject?

Air pollution is an environmental risk factor of concern in urban centers all over the world. Reduction in secondary sex ratio has been suggested to be indicative of potential influences of polluted environments on reproductive function. Previous study analyzed the relationship between air pollution and secondary sex ratio in an urban center of a developing country in a restricted time series period.

What does this study add?

The pollution levels in the city has declined significantly due to the national pollution control program since previous evaluation; thus this study extended the period analysis in order to assess if changes in the particulate matter concentration are followed by changes in secondary sex ratio. Data have shown a strong association and could indicate SSR as a potential indicator of population health status orientating future public policies for environmental control.

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Contributorship Statement

The corresponding author has conceived and planned the study design and analysis; all the authors have contributed with the analysis, elaboration and final approval of the manuscript.

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INTRODUCTION

Air pollution is an environmental risk factor of concern in urban centers all over the world. Respiratory and cardiovascular diseases are the most commonly observed and associated effects followed by neoplasia,[1, 2, 3]. However, in the last two decades lesser known effects associated to chronic air pollution exposures have started to emerge [4]. New epidemiological and experimental studies link exposure to reproductive adverse outcomes and investigations have risen different effects to be attributed to air pollution such as low birth weight,[5] miscarriages, [6], preterm birth [7] and decrease sperm quality [8].

Secondary sex ratio (number of male births in relation to total births) seems to be affected in population living in polluted environments and occupationally exposed to certain chemicals,[9,10,11,12,13]. Although the causality between environmental exposures and declines in secondary sex ration are still controversial, some authors suggest that the SSR as a sentinel indicator of reproductive injury and avoidable health exposures,[14] due to environmental pollution.

Experimental evidence indicate that prenatal exposure to air pollution derived from diesel exhausts is associated with altered sexual differentiation and function,[15]. Studies in humans and animals have found a reduction in the number of male births associated with lower male fertility, but the mechanism by which environmental hazards might change the sex ratio has not yet been established [9,16].

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In a previous study, we have demonstrated a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}), [16]. This study was conducted in São Paulo Metropolitan Region (SPMR) in Brazil, and the area was divided in terms of level of PM_{10} concentrations. Findings indicated a SSR of 51.7% for the less polluted area whereas for highly polluted area the ratio decreased to 50.7%. This result corresponds to a difference of 1% in total male births, or 1,180 fewer male births in the most polluted regions),[16].

Previous data analyzed a restricted time series period (2001-2003) and during the last years air pollution levels in the city has changed significantly due to the national pollution control program (PROCONVE and PROMOT). In this sense, it is desirable to verify if changes in levels of air pollution are accompanied by concurrent changes in the SSR in SPMR. Thus, the purpose of this study was to extend the time period evaluating from 2000 to 2007 to assess changes in ambient particulate matter (PM_{10}) concentrations and secondary sex ratio in the RMSP during this period (SRR).

METHODS

Number of births according to gender

The total number of live births in São Paulo was collected from 2000 to 2007 in a monthly basis representing a sample of 53,612 births. These records were obtained from SEADE, a public foundation which registers population data in the State of São Paulo. The male ratio was chosen to represent the secondary sex ratio (SSR=total male birth/total births).

Studied Area

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São Paulo is the largest Brazilian city, where most important economic activity is concentrated and is responsible for 17% of the country's gross national product. São Paulo is considered the 6^{th} largest city in the world with a population of approximately 11 million in an area of 7,943.82 km² [17].

According to the São Paulo's Environmental State Agency air pollution is derived mostly by vehicles (combustion and re-suspension) and a small industrial contribution. Winter period in São Paulo favors thermal inversions and this may also contribute to non-favorable pollutant dispersion scenario and increased levels of PM_{10} [18]. Air pollution control programs in São Paulo Metropolitan Area are well succeed for the fixed sources however the mobile sources are of government concern.

Air pollution Data

The studied area encompasses the sub-districts where the state environmental agency (CETESB) has air monitoring stations, and were selected according to different air pollution gradients (high and low concentrations' areas). In the case of São Paulo we have a well spread air pollution monitoring system thus we assumed that the concentration of a given region would reflect somehow the exposure. Since we have information about birth outcomes aggregated by administrative districts in São Paulo, we assumed that the station located in a given districted would reflect the exposure of pregnant women living in that given district. We did not have access to information about maternal mobility during gestation. We assumed that pollution affects the mothers independently. We included districts for which we had good quality representative data (valid time series) and stratified according to the PM_{10} levels. The districts were aggregated according to the level of PM_{10} concentration as follow: high level ($\geq$40

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μg/m^3) and low level (< 40 μg/m^3). PM_{10} concentrations were used as a marker of overall air pollution.

In total data were obtained from 5 automatic monitoring stations maintained by CETESB. In all stations, PM_{10} was measured through inter compared beta radiation monitors. The daily values obtained from each station were averaged in a monthly basis and considered as indicative of city-wide pollution levels. There is a correlation between PM_{10} concentrations registered at the different sites that means that PM_{10} is regularly distributed along the citywide.

Statistical analysis

The SSR data from each area were analyzed according to the time variation and PM_{10} concentration in the areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

RESULTS

The sub district's average concentrations of PM_{10} in the period ranged from 34.1 μg/m^3 to 64μg/m^3 and the SSR from 0.49 to 0.52 as depicted in Fig. 1.

In the less polluted area, the SSR average was 51.4% for 28,022 births recorded whereas in the most polluted area the ratio decreased to 50.7 % for 22,590 births recorded. We observed a general decrease trend in PM_{10} concentrations through the analyzed time period while the SSR simultaneously presented an increase.

An analysis of percentage variations considering the extreme years of the time-series analysis (that is, 2007 compared to 2001) was conducted showing a continuous decrease of PM_{10} concentration associated to an increase in SSR in each monitoring

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sub-district in the period, except for one monitoring station, which presented the same average level (Fig. 2). Surprisingly, Cambuci (CBC) monitoring station presented no variation in both variables (PM_{10} and SSR); however, this finding confirms the association observed in the other stations where lower PM_{10} concentrations are related to higher SSR.

The exponential regression showed a negative and significant association between PM_{10} and SSR (Table 1).

Table 1. Bivariate exponential regression analysis and relative risk.

Variable	R²	β	p-value	RR
SSR	0,322	-0.001	0.022	0,999

SSR: Secundary Sex Ratio; RR: Relative Risk

Fig. 3 emphasizes the inversely relationship of PM_{10} concentrations and SSR, specially from 2002 on, when we can observe the annual variations in both variables occurring in opposite directions, reinforcing the above demonstrated findings.

DISCUSSION

In this study we have evaluated the variation in PM_{10} environmental concentration and SSR in the Metropolitan Region of Sao Paulo, Brazil during the years of 2001-2007. In a previous study conducted in the same area we have noted that there was a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}),[16]. In this study we extended analyzed time period, which allowed us to observe improvements in air quality due to the environmental control politics introduced (motorized vehicles' emissions control) and in

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the population's gender pattern. Although the air quality increased we still find a significant negative association between the SSR and PM_{10} concentration.

Assessments of emissions source of the particulate air pollution in São Paulo city conducted by CETESB (São Paulo Environmental State Agency) [18] and several studies conducted in São Paulo [19, 20], using the receptor models and chemical comprehensive characterization of particles have indicated that 90% of PM_{10} is generated by vehicles or photochemical process. PM_{10} should not be considered a single pollutant; it is a synthesis of air pollutants, carrying primary and secondary pollutants, its composition includes carbon and many other chemicals depending on its emission source. In the referred stations there were an improvement of the diesel fuel and motors' technology, added by a traffic detour due to an implementation of a road infrastructure (this behavior was observed in PDP station).

In one region of the city, where CBC station is located (central area of São Paulo city) no variation in PM_{10} was noted and we can speculate that this no variation in PM_{10} is due by the fact that this area has buses emissions as main air pollution source, with lower contributions from cars and motorcycles. No variations in PM_{10} in this region shows that air pollution control program have not positively impacted the area leading to the maintenance of the air pollution level. Maintenance of PM_{10} levels was accompanied by maintenance of the SSR for this region. CBC station records and associated SSR can be interpreted as a control unit for other stations where there were variations in PM_{10} concentration meaning that for the same level of air pollution the same SSR was registered.

These results could suggest that there is a possible contribution of PM_{10} levels in SSR variation, explaining more than 30% of the events. If we consider that there is causal relationship the increase of 10μg/m³ in PM_{10} concentration would lead to a

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decline of 0.995% in SSR. Further, taking into account a PM_{10} average concentration in São Paulo city of 44.72 μg/m^3 in this study period the SSR decline would reach almost 4.37% which is equivalent to 30,934 less male births.

This behavior (decrease in PM_{10} and increase in SSR) is consistent with previous findings ,[15] that have shown a possible association between exposure to urban air pollution and imbalance of the sex ratio at birth. Other studies have also reported lower sex ratio in residential areas at risk from air pollution emitted from incinerators [21] as well as higher sex ratio in areas exposed to polluted air from steel foundry [22].

In humans the sex of the baby is determined primarily by the fecundation of the X egg by the X (female) or Y (male) sperm. In the case of environmental exposures and changes in the secondary sex ratio as a health outcome, it is very difficult to determine the time connection between gender at birth because the effect could have occurred even before pregnancy. Further, changes in the sex ratio may be associated with maternal or paternal factors or with both. Pre implantation hypothesis proposes that in some circumstances there are more favorable development or survival of X or Y bearing sperm or survival of male or female embryos [23-25]. In a previous study of our group we have shown that exposure to PM during the preconception period are associated to early pregnancy loss in women undergoing in vitro fertilization [26] and thus there is also another possibility to explain the changes in sex ratio by sex specific increases in intrauterine death or stillbirth.

Potential toxicological mechanisms that might explain and give strength to the environmental contamination causes in the determination of the sex ratio are still inconclusive. There are some suggestions in the literature that include the hormonal status of the parents at the time of conception, differential characteristics and sensibility of sperm of one sex, combination and presence of specific toxic substances (PAH,

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dioxin) [27,28]. Although we have not evaluated the elemental composition of PM10, previous studies have characterized the composition of these particles from São Paulo city. Chemical elements included Fe, Br, Al, Si, S, Cu, Zn, Pb [29] and PAH such as benzene, toluene, etil-benzene e xylene [30]. Toxicological studies have shown that certain toxicants present in ambient air pollution, such as PAH and heavy metals potential endocrine disruptors [31,32].

This is a descriptive study which does not intend to implicate in causality and it subsidizes on a previous research [16]. The changes in air pollution were compatible with the effects' variation and there is a toxicological support for that [16]. In this sense, it is a limitation but once it is a trend study and the measures to be aggregated are monthly records (SSR) and daily measures (PM_{10}), a synchrony between exposure and gender determination is minimized when you aggregate data on yearly basis. This is a different situation from a classical time series study because you know exactly the time relationship between exposure and health outcome (death or hospital admission). In the case of considering SSR as a health outcome, it is very difficult to determine the time connection between exposure and sex at birth; as previously demonstrated it can occur before conception, during embryonic implantation or gestation. When you aggregate the data in a yearly basis you encompass these phases, therefore in a times series study we can not capture this effect. It could be done in a birth cohort study but once these prematurity are scarce events, the size of the sample would become this a complex and costly study.

Increasing differences in the male/female ratio at birth could lead, in a mid-long term future, to a deficit in male's population and probably cause social problems. This scenario gets worst if we consider that male are more prone to premature death because of their trend to engage in risk behavior and violence,[33].

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The air pollution control programs (PROCONVE and PROMOT, which refers to emissions limit to new motor vehicles – cars and motorbikes) may have contributed to the improvement in the air quality parameters registered through the decade. Recently, an inspection and maintenance program concerning emissions limits for the old and second handed vehicles was implemented in São Paulo and that may also have favored this scenario. Our findings are important indicators for an advance of the public health endpoints due to the improvement of the air quality in urban centers. Considering the disproportion in the male/female births, this balance is desirable to achieve and maintain in all populations of urban centers. Furthermore, the abatement of air pollution is a target that governments must pursue.

CONCLUSIONS

Although the biological mechanisms responsible for the SSR changes are not clearly established, this study indicate that concentration of particulate air pollution in urban cities are associated with decreased SSR. Also, this data give support for the use of SSR as a potential indicator of the negative health impacts of fuels combustion derived emissions in urban cities.

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Competing Interests

None

Data sharing

There are no additional unplublished data of the study to be shared.

Contributorship

Mariana Veras has contributed to the Introduction Section. Simone Miraglia, Fernando Rodrigues-Silva, Luis Amato-Lourenço and Paulo Saldiva has conducted the data collection and statistical analysis of the research. Simone Miraglia has defined the study design.

All authors have contributed to the revised final version of the manuscript and to the discussion section.

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REFERENCES

1. Schwartz J.. Long-term effects of exposure to particulate air pollution. *Clin Occup Environ Med* 2006,5(4):837-848.

2. Pope CA, 3rd, Ezzati M, Dockery DW.. Fine-particulate air pollution and life expectancy in the United States. *The New England journal of medicine* 2009,360(4): 376-386.

3. Dockery DW. Health effects of particulate air pollution. *Ann Epidemiol* 2009,19(4): 257-263.

4. Calderon-Garciduenas L, Engle R, Mora-Tiscareno A et al. Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and cognition* 2011,77(3): 345-355.

5. Gouveia N, Bremner SA, Novaes HMD. Association between ambient air pollution and birth weight in São Paulo, Brazil. *Journal of Epidemiology and Community Health* 2004,58(1): 11-17.

6. Mohorovic L, Petrovic O, Haller H et al. Pregnancy loss and maternal methemoglobin levels: an indirect explanation of the association of environmental toxics and their adverse effects on the mother and the fetus. Int J Environ Res Public Health.2010 Dec;7(12):4203-12. Epub 2010.

7. van den Hooven EH, Pierik FH, de Kluizenaar Y et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environmental health perspectives* 2012,120(1): 150-156.

8. Pires A, de Melo EN, Mauad T et al. Pre- and postnatal exposure to ambient levels of urban particulate matter (PM(2.5)) affects mice spermatogenesis. *Inhal Toxicol* 2011,23(4): 237-245.

9. Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. *Emerging Health Threats Journal* 2011;Vol 4 (2011) incl Supplements.

10. Tragaki A, Lasaridi K. Temporal and spatial trends in the sex ratio at birth in Greece, 1960–2006: exploring potential environmental factors. *Population & Environment* 2009,30(3): 114-128.

11. Schnorr TM, Lawson CC, Whelan EA et al. Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environmental health perspectives* 2001,109(11): 1127-1132.

12. Ryan JJ, Amirova Z, Carrier G.Sex ratios of children of Russian pesticideproducers exposed to dioxin. EnvironHealth Perspect. 2002;110(11):A699-701.

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13. Yang CY, Tsai SS, Cheng BH et al. Sex Ratio at Birth Associated with Petrochemical Air Pollution in Taiwan. *Bulletin of Environmental Contamination and Toxicology* 2000,65(1): 126-131.

14. Davis DL, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA : the journal of the American Medical Association* 1998,279(13): 1018-1023.

15. Watanabe N, Kurita M. The Masculinization of the Fetus During Pregnancy Due to Inhalation of Diesel Exhaust. *Environmental health perspectives* 2001,109(2).

16. Lichtenfels AJ, Gomes JB, Pieri PC et al. Increased levels of air pollution and a decrease in the human and mouse male-to-female ratio in Sao Paulo, Brazil. *Fertil Steril* 2007,87(1): 230-232.

17. Fundação Seade. http://www.seade.gov.br/produtos/pib/index.php Acessed on 08/07/2012.

18. CETESB. Qualidade do ar no estado de São Paulo 2011. São Paulo : CETESB, 2012.

19. Miranda, R. M**.** ; Andrade, M. F. ; Fornaro, A. ; et al. Urban air pollution: a representative survey of PM2.5 mass concentrations in six Brazilian cities. Air quality Atm and Health, v. 5, p. 63, 2012.

20. Andrade, M. F. ; Miranda, R. M**.** ; Fornaro, A. et al. Vehicle emissions and PM2.5 mass concentrations in six Brazilian cities. Air quality atmosphere and health, v. 5, p. 79, 2012.

21. Williams FL, Lawson AB, Lloyd OL. Low sex ratios of births in areas at risk from air pollution from incinerators, as shown by geographical analysis and 3-dimensional mapping. Int J Epidemiol. 1992 ;21(2):311-9.

22. Lloyd OL, Smith G, Lloyd MM et al.. Raised mortality from lung cancer and high sex ratios of births associated with industrial pollution. Br J Ind Med. 1985 ;42(7):475-80.

23. Mocarelli P, Gerthoux PM, Patterson DG Jr, et al. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ Health Perspect. 2008;116(1):70-7.

24. Hansen D, Moller H and Olsen J Severe periconceptional life events and the sex ratio in offspring: follow up study based on five national registers. Br Med J 1999; 319:548–549.

25. Boklage CE.The epigenetic environment: secondary sex ratio depends on differential survival in embryogenesis. Hum Reprod. 2005 Mar;20(3):583-7.

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26. Perin PM, Maluf M, Czeresnia CE, et al. . Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). J Assist Reprod Genet. 2010; 27(7):371-82.

27. James WH. Hypotheses on the stability and variation of human sex ratios at birth. J Theor Biol. 2012;310:183-6. doi: 10.1016/j.jtbi.2012.06.038. Epub 2012 Jul 7.

28. James WH. The categories of evidence relating to the hypothesis that mammalian sex ratios at birth are causally related to the hormone concentrations of both parents around the time of conception. J Biosoc Sci. 2011;43(2):167-84.

29. Sánchez-Ccoyllo OR, Ynoue RY, Martins LD et al.. Vehicular particulate matter emissions in road tunnels in São Paulo, Brazil. Environ Monit Assess 2009; 149:241-9.

30. Carvalho-Oliveira A, Pozo RMK, Lobo DJA et al. Diesel emissions significantly influence composition and mutagenicity of ambient particles: a case study in São Paulo, Brazil. Environ Res 2005; 98:1-7.

31. Mattison, D.R.,Thomford,P.J.,1989.The mechanisms of action of reproductive toxicants.Toxicol.Pathol.17,364–376.

32. Borman, S.M., Christian,P.J.,Sipes,I.G. et al.Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol.Appl.Pharmacol.2000 - 167,191–198.

33. Waldron I. Recent trends in sex mortality ratios for adults in developed countries. *Soc Sci Med* 1993,36(4): 451-462.

LIST OF FIGURES

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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil - a times series study.

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The pollution levels in the city has declined significantly due to the national pollution control program since previous evaluation; thus this study extended the period analysis in order to assess if changes in the particulate matter concentration are followed by changes in secondary sex ratio. Data have shown a strong association and could indicate SSR as a potential indicator of population health status orientating future public policies for environmental control.

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Contributorship Statement

The corresponding author has conceived and planned the study design and analysis; all the authors have contributed with the analysis, elaboration and final approval of the manuscript.

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INTRODUCTION

Air pollution is an environmental risk factor of concern in urban centers all over the world. Respiratory and cardiovascular diseases are the most commonly observed and associated effects followed by neoplasia,[1, 2, 3]. However, in the last two decades lesser known effects associated to chronic air pollution exposures have started to emerge [4]. New epidemiological and experimental studies link exposure to reproductive adverse outcomes and investigations have risen different effects to be attributed to air pollution such as low birth weight,[5] miscarriages, [6], preterm birth [7] and decrease sperm quality [8].

Secondary sex ratio (number of male births in relation to total births) seems to be affected in population living in polluted environments and occupationally exposed to certain chemicals,[9,10,11,12,13]. Although the causality between environmental exposures and declines in secondary sex ration are still controversial, some authors suggest that the SSR as a sentinel indicator of reproductive injury and avoidable health exposures,[14] due to environmental pollution.

Experimental evidence indicate that prenatal exposure to air pollution derived from diesel exhausts is associated with altered sexual differentiation and function,[15]. Studies in humans and animals have found a reduction in the number of male births associated with lower male fertility, but the mechanism by which environmental hazards might change the sex ratio has not yet been established [9,16].

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In a previous study, we have demonstrated a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}), [16]. This study was conducted in São Paulo Metropolitan Region (SPMR) in Brazil, and the area was divided in terms of level of PM_{10} concentrations. Findings indicated a SSR of 51.7% for the less polluted area whereas for highly polluted area the ratio decreased to 50.7%. This result corresponds to a difference of 1% in total male births, or 1,180 fewer male births in the most polluted regions),[16].

Previous data analyzed a restricted time series period (2001-2003) and during the last years air pollution levels in the city has changed significantly due to the national pollution control program (PROCONVE and PROMOT). In this sense, it is desirable to verify if changes in levels of air pollution are accompanied by concurrent changes in the SSR in SPMR. Thus, the purpose of this study was to extend the time period evaluating from 2000 to 2007 to assess changes in ambient particulate matter (PM_{10}) concentrations and secondary sex ratio in the RMSP during this period (SRR).

METHODS

Number of births according to gender

The total number of live births in São Paulo was collected from 2000 to 2007 in a monthly basis representing a sample of 53,612 births. These records were obtained from SEADE, a public foundation which registers population data in the State of São Paulo. The male ratio was chosen to represent the secondary sex ratio (SSR=total male birth/total births).

Studied Area

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São Paulo is the largest Brazilian city, where most important economic activity is concentrated and is responsible for 17% of the country's gross national product. São Paulo is considered the 6th largest city in the world with a population of approximately 11 million in an area of 7,943.82 km² [17].

According to the São Paulo's Environmental State Agency air pollution is derived mostly by vehicles (combustion and re-suspension) and a small industrial contribution. Winter period in São Paulo favors thermal inversions and this may also contribute to non-favorable pollutant dispersion scenario and increased levels of PM_{10} [18]. Air pollution control programs in São Paulo Metropolitan Area are well succeed for the fixed sources however the mobile sources are of government concern.

Air pollution Data

The studied area encompasses the sub-districts where the state environmental agency (CETESB) has air monitoring stations, and were selected according to different air pollution gradients (high and low concentrations' areas). In the case of São Paulo we have a well spread air pollution monitoring system thus we assumed that the concentration of a given region would reflect somehow the exposure. Since we have information about birth outcomes aggregated by administrative districts in São Paulo, we assumed that the station located in a given districted would reflect the exposure of pregnant women living in that given district. We did not have access to information about maternal mobility during gestation. We assumed that pollution affects the mothers independently. We included districts for which we had good quality representative data (valid time series) and stratified according to the PM_{10} levels. The districts were aggregated according to the level of PM_{10} concentration as follow: high level (≥40

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μg/m^3) and low level (< 40 μg/m^3). PM_{10} concentrations were used as a marker of overall air pollution.

In total data were obtained from 5 automatic monitoring stations maintained by CETESB. In all stations, PM_{10} was measured through inter compared beta radiation monitors. The daily values obtained from each station were averaged in a monthly basis and considered as indicative of city-wide pollution levels. There is a correlation between PM_{10} concentrations registered at the different sites that means that PM_{10} is regularly distributed along the citywide.

Statistical analysis

The SSR data from each area were analyzed according to the time variation and PM_{10} concentration in the areas using descriptive statistics. The strength association between annual average of PM_{10} concentration and SSR was performed through exponential regression, and it was adopted a statistical significance level of $p<0.05$.

RESULTS

The sub district's average concentrations of PM_{10} in the period ranged from 34.1 μg/m^3 to 64μg/m^3 and the SSR from 0.49 to 0.52 as depicted in Fig. 1.

In the less polluted area, the SSR average was 51.4% for 28,022 births recorded whereas in the most polluted area the ratio decreased to 50.7 % for 22,590 births recorded. We observed a general decrease trend in PM_{10} concentrations through the analyzed time period while the SSR simultaneously presented an increase.

An analysis of percentage variations considering the extreme years of the time-series analysis (that is, 2007 compared to 2001) was conducted showing a continuous decrease of PM_{10} concentration associated to an increase in SSR in each monitoring

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sub-district in the period, except for one monitoring station, which presented the same average level (Fig. 2). Surprisingly, Cambuci (CBC) monitoring station presented no variation in both variables (PM_{10} and SSR); however, this finding confirms the association observed in the other stations where lower PM_{10} concentrations are related to higher SSR.

The exponential regression showed a negative and significant association between PM_{10} and SSR (Table 1).

Table 1. Bivariate exponential regression analysis and relative risk.

Variable	R²	β	p-value	RR
SSR	0,322	-0.001	0.022	0,999

SSR: Secundary Sex Ratio; RR: Relative Risk

Fig. 3 emphasizes the inversely relationship of PM_{10} concentrations and SSR, specially from 2002 on, when we can observe the annual variations in both variables occurring in opposite directions, reinforcing the above demonstrated findings.

DISCUSSION

In this study we have evaluated the variation in PM_{10} environmental concentration and SSR in the Metropolitan Region of Sao Paulo, Brazil during the years of 2001-2007. In a previous study conducted in the same area we have noted that there was a significant negative association between the sex ratio at birth or SSR and ambient levels of particulate matter (PM_{10}),[16]. In this study we extended analyzed time period, which allowed us to observe improvements in air quality due to the environmental control politics introduced (motorized vehicles' emissions control) and in

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the population's gender pattern. Although the air quality increased we still find a significant negative association between the SSR and PM_{10} concentration.

Assessments of emissions source of the particulate air pollution in São Paulo city conducted by CETESB (São Paulo Environmental State Agency) [18] and several studies conducted in São Paulo [19, 20], using the receptor models and chemical comprehensive characterization of particles have indicated that 90% of PM_{10} is generated by vehicles or photochemical process. PM_{10} should not be considered a single pollutant; it is a synthesis of air pollutants, carrying primary and secondary pollutants, its composition includes carbon and many other chemicals depending on its emission source. In the referred stations there were an improvement of the diesel fuel and motors' technology, added by a traffic detour due to an implementation of a road infrastructure (this behavior was observed in PDP station).

In one region of the city, where CBC station is located (central area of São Paulo city) no variation in PM_{10} was noted and we can speculate that this no variation in PM_{10} is due by the fact that this area has buses emissions as main air pollution source, with lower contributions from cars and motorcycles. No variations in PM_{10} in this region shows that air pollution control program have not positively impacted the area leading to the maintenance of the air pollution level. Maintenance of PM_{10} levels was accompanied by maintenance of the SSR for this region. CBC station records and associated SSR can be interpreted as a control unit for other stations where there were variations in PM_{10} concentration meaning that for the same level of air pollution the same SSR was registered.

These results could suggest that there is a possible contribution of PM_{10} levels in SSR variation, explaining more than 30% of the events. If we consider that there is causal relationship the increase of 10μg/m³ in PM_{10} concentration would lead to a

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decline of 0.995% in SSR. Further, taking into account a PM_{10} average concentration in São Paulo city of 44.72 µg/m^3 in this study period the SSR decline would reach almost 4.37% which is equivalent to 30,934 less male births.

This behavior (decrease in PM_{10} and increase in SSR) is consistent with previous findings ,[15] that have shown a possible association between exposure to urban air pollution and imbalance of the sex ratio at birth. Other studies have also reported lower sex ratio in residential areas at risk from air pollution emitted from incinerators [21] as well as higher sex ratio in areas exposed to polluted air from steel foundry [22].

In humans the sex of the baby is determined primarily by the fecundation of the X egg by the X (female) or Y (male) sperm. In the case of environmental exposures and changes in the secondary sex ratio as a health outcome, it is very difficult to determine the time connection between gender at birth because the effect could have occurred even before pregnancy. Further, changes in the sex ratio may be associated with maternal or paternal factors or with both. Pre implantation hypothesis proposes that in some circumstances there are more favorable development or survival of X or Y bearing sperm or survival of male or female embryos [23-25]. In a previous study of our group we have shown that exposure to PM during the preconception period are associated to early pregnancy loss in women undergoing in vitro fertilization [26] and thus there is also another possibility to explain the changes in sex ratio by sex specific increases in intrauterine death or stillbirth.

Potential toxicological mechanisms that might explain and give strength to the environmental contamination causes in the determination of the sex ratio are still inconclusive. There are some suggestions in the literature that include the hormonal status of the parents at the time of conception, differential characteristics and sensibility of sperm of one sex, combination and presence of specific toxic substances (PAH,

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dioxin) [27,28]. Although we have not evaluated the elemental composition of PM10, previous studies have characterized the composition of these particles from São Paulo city. Chemical elements included Fe, Br, Al, Si, S, Cu, Zn, Pb [29] and PAH such as benzene, toluene, etil-benzene e xylene [30]. Toxicological studies have shown that certain toxicants present in ambient air pollution, such as PAH and heavy metals potential endocrine disruptors [31,32].

This is a descriptive study which does not intend to implicate in causality and it subsidizes on a previous research [16]. The changes in air pollution were compatible with the effects' variation and there is a toxicological support for that [16]. In this sense, it is a limitation but once it is a trend study and the measures to be aggregated are monthly records (SSR) and daily measures (PM_{10}), a synchrony between exposure and gender determination is minimized when you aggregate data on yearly basis. This is a different situation from a classical time series study because you know exactly the time relationship between exposure and health outcome (death or hospital admission). In the case of considering SSR as a health outcome, it is very difficult to determine the time connection between exposure and sex at birth; as previously demonstrated it can occur before conception, during embryonic implantation or gestation. When you aggregate the data in a yearly basis you encompass these phases, therefore in a times series study we can not capture this effect. It could be done in a birth cohort study but once these prematurity are scarce events, the size of the sample would become this a complex and costly study.

Increasing differences in the male/female ratio at birth could lead, in a mid-long term future, to a deficit in male's population and probably cause social problems. This scenario gets worst if we consider that male are more prone to premature death because of their trend to engage in risk behavior and violence,[33].

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The air pollution control programs (PROCONVE and PROMOT, which refers to emissions limit to new motor vehicles – cars and motorbikes) may have contributed to the improvement in the air quality parameters registered through the decade. Recently, an inspection and maintenance program concerning emissions limits for the old and second handed vehicles was implemented in São Paulo and that may also have favored this scenario. Our findings are important indicators for an advance of the public health endpoints due to the improvement of the air quality in urban centers. Considering the disproportion in the male/female births, this balance is desirable to achieve and maintain in all populations of urban centers. Furthermore, the abatement of air pollution is a target that governments must pursue.

CONCLUSIONS

Although the biological mechanisms responsible for the SSR changes are not clearly established, this study indicate that concentration of particulate air pollution in urban cities are associated with decreased SSR. Also, this data give support for the use of SSR as a potential indicator of the negative health impacts of fuels combustion derived emissions in urban cities.

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REFERENCES

1. Schwartz J.. Long-term effects of exposure to particulate air pollution. *Clin Occup Environ Med* 2006,5(4):837-848.

2. Pope CA, 3rd, Ezzati M, Dockery DW.. Fine-particulate air pollution and life expectancy in the United States. *The New England journal of medicine* 2009,360(4): 376-386.

3. Dockery DW. Health effects of particulate air pollution. *Ann Epidemiol* 2009,19(4): 257-263.

4. Calderon-Garciduenas L, Engle R, Mora-Tiscareno A et al. Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and cognition* 2011,77(3): 345-355.

5. Gouveia N, Bremner SA, Novaes HMD. Association between ambient air pollution and birth weight in São Paulo, Brazil. *Journal of Epidemiology and Community Health* 2004,58(1): 11-17.

6. Mohorovic L, Petrovic O, Haller H et al. Pregnancy loss and maternal methemoglobin levels: an indirect explanation of the association of environmental toxics and their adverse effects on the mother and the fetus. Int J Environ Res Public Health.2010 Dec;7(12):4203-12. Epub 2010.

7. van den Hooven EH, Pierik FH, de Kluizenaar Y et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environmental health perspectives* 2012,120(1): 150-156.

8. Pires A, de Melo EN, Mauad T et al. Pre- and postnatal exposure to ambient levels of urban particulate matter (PM(2.5)) affects mice spermatogenesis. *Inhal Toxicol* 2011,23(4): 237-245.

9. Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. *Emerging Health Threats Journal* 2011;Vol 4 (2011) incl Supplements.

10. Tragaki A, Lasaridi K. Temporal and spatial trends in the sex ratio at birth in Greece, 1960–2006: exploring potential environmental factors. *Population & Environment* 2009,30(3): 114-128.

11. Schnorr TM, Lawson CC, Whelan EA et al. Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environmental health perspectives* 2001,109(11): 1127-1132.

12. Ryan JJ, Amirova Z, Carrier G.Sex ratios of children of Russian pesticideproducers exposed to dioxin. EnvironHealth Perspect. 2002;110(11):A699-701.

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13. Yang CY, Tsai SS, Cheng BH et al. Sex Ratio at Birth Associated with Petrochemical Air Pollution in Taiwan. *Bulletin of Environmental Contamination and Toxicology* 2000,65(1): 126-131.

14. Davis DL, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA : the journal of the American Medical Association* 1998,279(13): 1018-1023.

15. Watanabe N, Kurita M. The Masculinization of the Fetus During Pregnancy Due to Inhalation of Diesel Exhaust. *Environmental health perspectives* 2001,109(2).

16. Lichtenfels AJ, Gomes JB, Pieri PC et al. Increased levels of air pollution and a decrease in the human and mouse male-to-female ratio in Sao Paulo, Brazil. *Fertil Steril* 2007,87(1): 230-232.

17. Fundação Seade. http://www.seade.gov.br/produtos/pib/index.php Acessed on 08/07/2012.

18. CETESB. Qualidade do ar no estado de São Paulo 2011. São Paulo : CETESB, 2012.

19. Miranda, R. M**.** ; Andrade, M. F. ; Fornaro, A. ; et al. Urban air pollution: a representative survey of PM2.5 mass concentrations in six Brazilian cities. Air quality Atm and Health, v. 5, p. 63, 2012.

20. Andrade, M. F. ; Miranda, R. M**.** ; Fornaro, A. et al. Vehicle emissions and PM2.5 mass concentrations in six Brazilian cities. Air quality atmosphere and health, v. 5, p. 79, 2012.

21. Williams FL, Lawson AB, Lloyd OL. Low sex ratios of births in areas at risk from air pollution from incinerators, as shown by geographical analysis and 3-dimensional mapping. Int J Epidemiol. 1992 ;21(2):311-9.

22. Lloyd OL, Smith G, Lloyd MM et al.. Raised mortality from lung cancer and high sex ratios of births associated with industrial pollution. Br J Ind Med. 1985 ;42(7):475-80.

23. Mocarelli P, Gerthoux PM, Patterson DG Jr, et al. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environ Health Perspect. 2008;116(1):70-7.

24. Hansen D, Moller H and Olsen J Severe periconceptional life events and the sex ratio in offspring: follow up study based on five national registers. Br Med J 1999; 319:548–549.

25. Boklage CE.The epigenetic environment: secondary sex ratio depends on differential survival in embryogenesis. Hum Reprod. 2005 Mar;20(3):583-7.

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26. Perin PM, Maluf M, Czeresnia CE, et al. . Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). J Assist Reprod Genet. 2010; 27(7):371-82.

27. James WH. Hypotheses on the stability and variation of human sex ratios at birth. J Theor Biol. 2012;310:183-6. doi: 10.1016/j.jtbi.2012.06.038. Epub 2012 Jul 7.

28. James WH. The categories of evidence relating to the hypothesis that mammalian sex ratios at birth are causally related to the hormone concentrations of both parents around the time of conception. J Biosoc Sci. 2011;43(2):167-84.

29. Sánchez-Ccoyllo OR, Ynoue RY, Martins LD et al.. Vehicular particulate matter emissions in road tunnels in São Paulo, Brazil. Environ Monit Assess 2009; 149:241-9.

30. Carvalho-Oliveira A, Pozo RMK, Lobo DJA et al. Diesel emissions significantly influence composition and mutagenicity of ambient particles: a case study in São Paulo, Brazil. Environ Res 2005; 98:1-7.

31. Mattison, D.R.,Thomford,P.J.,1989.The mechanisms of action of reproductive toxicants.Toxicol.Pathol.17,364–376.

32. Borman, S.M., Christian,P.J.,Sipes,I.G. et al.Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. Toxicol.Appl.Pharmacol.2000 - 167,191–198.

33. Waldron I. Recent trends in sex mortality ratios for adults in developed countries. *Soc Sci Med* 1993,36(4): 451-462.

LIST OF FIGURES

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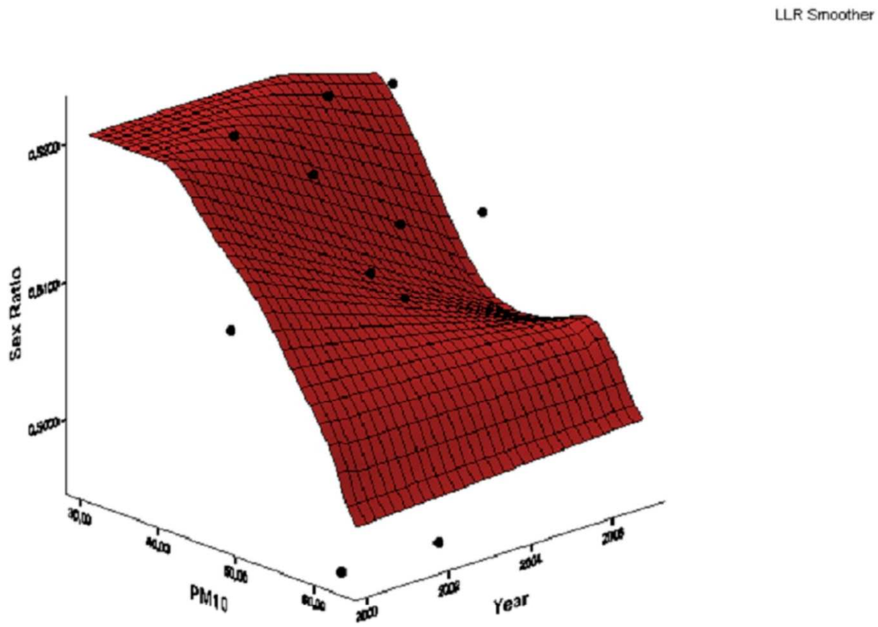

Fig. 1. Relation between Sex Ratio and PM10 in the period (2000-2007)
110x90mm (300 x 300 DPI)

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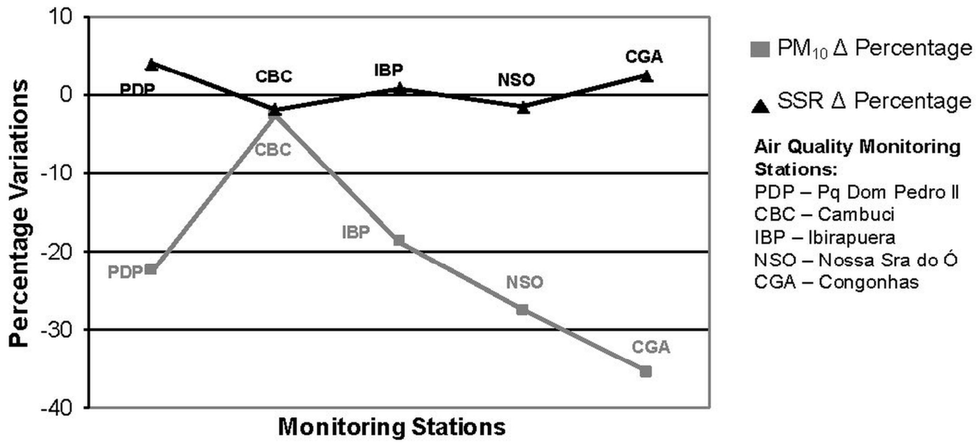

Fig. 2. PM10 and SSR percentage variations in the period (2000-2007) for the different monitoring station.
187x90mm (300 x 300 DPI)

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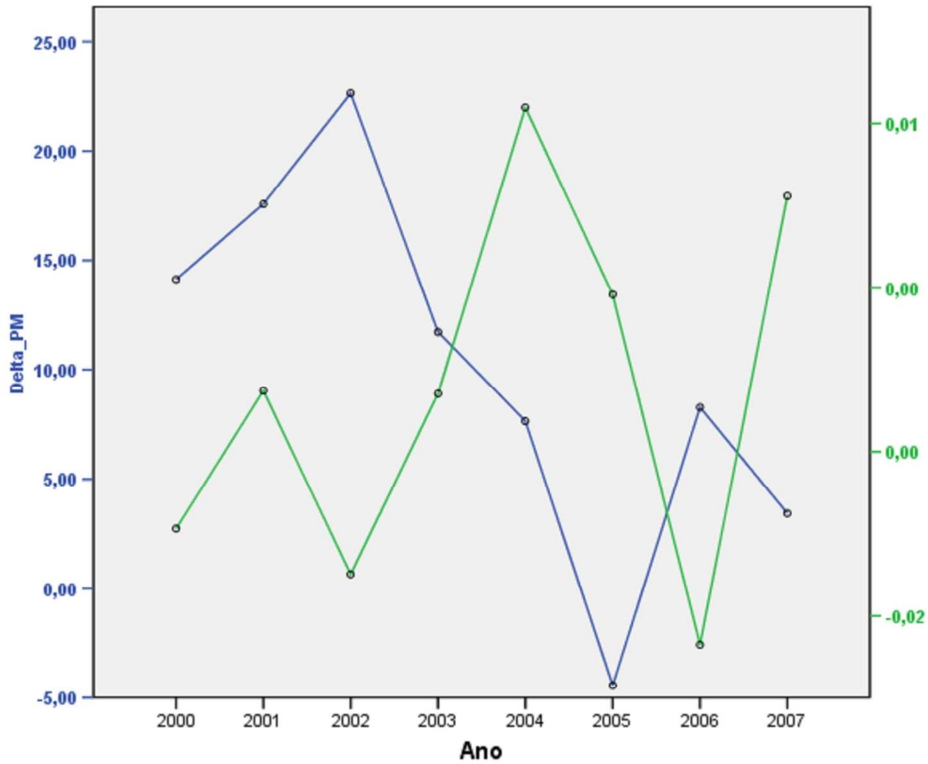

Fig. 3. Delta PM10 and delta SSR percentage variations along the analyzed period (2000-2007)
112x90mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *times series studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Non applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	Non applicable
Study size	10	Explain how the study size was arrived at	Non applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Non applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	Non applicable
		(*c*) Explain how missing data were addressed	Non applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Non applicable
		(*e*) Describe any sensitivity analyses	Non applicable
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Non applicable
		(b) Give reasons for non-participation at each stage	Non applicable
		(c) Consider use of a flow diagram	Non applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Non applicable
		(b) Indicate number of participants with missing data for each variable of interest	Non applicable
Outcome data	15*	Report numbers of outcome events or summary measures	Non applicable
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	9-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Non applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Non applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Non applicable

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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Follow-up of the air pollution and the human male-to-female ratio analysis in São Paulo, Brazil: a times series study

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